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STUDIES IN BACILLUS WELCHII, WITH SPECIAL REFERENCE TO
CLASSIFICATION AND TO ITS RELATION TO DIARRHEA.

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STUDIES IN *BACILLUS WELCHII*, WITH SPECIAL REFERENCE
TO CLASSIFICATION AND TO ITS RELATION TO
DIARRHEA.*

BY J. P. SIMONDS, M.D.

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Boston.)

INTRODUCTION.

Bacillus welchii is a member of a populous and widely distributed species of bacteria which have in common the ability to ferment sugars with the production of butyric acid. The literature dealing with these bacteria is extensive, and, as Bredemann (37) remarks, "equally as great is the confusion which exists as a result of the sometimes quite superficial investigation and description of the bacteria belonging to the group."

The classification of the members of the group is a matter of difficulty. In the first place the copious terminology has been a source of no little confusion. No less than ten names have been given to as many strains which are either identical with or very closely related to *B. welchii*. This organism was first fully described in 1892 by Welch and Nuttall (386) under the name of *B. aerogenes capsulatus*. The following names applied to other similar organisms isolated by different investigators probably designate identical or closely related species: *Bacillus* of acute articular rheumatism of Achalme (1) (1891); *B. phlegmonis emphysematosæ* of Fraenkel (88) (1893); *B. enteritidis sporogenes* of Klein (179) (1895); *B. perfringens* of Veillon and Zuber (380) (1897); *B. emphysematis vaginæ* of Lindenthal (216) (1897); *B. cadaveris butyricus* of Buday (42) (1898); *Granulobacillus saccharobutyricus liquefaciens immobilis* of Schattenfroh and Grassberger (316) (1899). Migula (410) in 1900 attempted to apply his binomial terminology to this group and added confusion by giving the name *Bacterium emphysematosum* to *B. phlegmonis emphysematosæ* and *Bacterium welchii* to *B. aerogenes capsulatus*. Individual members of the group are now frequently designated "gas bacillus" (176, 389).

* Received for publication, June 27, 1914.

Schattenfroh and Grassberger (319), Sandler (308), and von Hibler (146) believe that *B. phlegmonis emphysematosæ* is merely a pathogenic form of the organism to which Schattenfroh and Grassberger gave the sesquipedalian title of *Granulobacillus saccharobutyricus liquefaciens immobilis*. This classification Fraenkel (91) resents. Hewlett (139) and Achalme (5) compared *B. enteritidis sporogenes* of Klein with the organism isolated by Achalme from a case of rheumatism and pronounced them identical. Achalme (5) also found *B. perfringens* identical with the last two organisms. Welch (389) and Fraenkel (90) concede the identity of *B. phlegmonis emphysematosæ* and *B. aerogenes capsulatus*. Jackson (164) made a comparative study of a strain of *B. enteritidis sporogenes* obtained from Professor Klein, and of the original strain of *B. aerogenes capsulatus* isolated by Welch and found them identical for all tests applied by him.

The literature abounds in contradictory statements concerning the biologic characteristics of these bacilli, especially with reference to the formation of indol and the production of toxins and hemolysins. Almost every investigator who has studied this group extensively has had to correct in later reports errors made in earlier ones. Thus Fraenkel at first stated that *B. phlegmonis emphysematosæ* produced no change in milk, but later found that under proper conditions it caused stormy fermentation of milk in the same manner as the other members of the group. The value of Klein's earlier work (179) is greatly reduced because he found after several years of investigation that most of his cultures had contained two organisms: *B. enteritidis sporogenes*, which Jackson proved to be identical with *B. aerogenes capsulatus*; and another spore-forming anaerobe, which he (Klein) named *B. cadaveris sporogenes* (184). Schattenfroh and Grassberger (318) refuse to recognize *B. butyricus* of Botkin (35) as a distinct species, stating it as their belief that his cultures contained a mixture of the true non-motile butyric acid bacillus (*B. welchii*) with some motile organism which liquefied casein.

CLASSIFICATION.

The group characteristics of these bacteria are the following: They are large, anthrax-like, Gram-positive, non-motile, anaerobic bacilli with slightly rounded ends, usually occurring singly or in pairs, rarely in short chains. Spore formation is inconstant and occurs only in alkaline media, never in pure cultures in media containing a fermentable sugar or free acid. They bring about the stormy fermentation of milk; that is, the milk is quickly coagulated and gas formation is so abundant as to break up the

curd and often to force parts of it above the cream ring. In milk cultures the odor of butyric acid is evident. Plain and sugar-free gelatin is liquefied very slowly or not at all. A rabbit injected intravenously with this organism, killed within two or three minutes and incubated, presents in twenty-four hours a body enormously distended with gas which will burn with a pale blue flame.

Members of this group, therefore, differ from the so called "motile butyric acid bacilli" on the one hand, and from the "putrefactive butyric acid bacilli" (the "*fäulniserregenden Buttersäurebazillen*" of Schattenfroh and Grassberger (320, 321)), on the other. The motile butyric acid bacilli, such as *B. amylobacter* of Gruber (412) and of Bredemann (37), *B. saccharobutyricus* of von Klecki (413), *Granulobacter butyricum* of Beijerinck (415), and the strain isolated from milk by Grassberger and Schattenfroh (113), all possess in common motility, ability to form spores in milk and other sugar-containing media, and, especially when grown on media containing starch, contain granules which stain blue with iodine (114). These characteristics differentiate them sharply from the *B. welchii* group, the members of which lack these qualities. *B. welchii* is, on the other hand, differentiated from the "putrefactive butyric acid bacilli," such as *B. chauvei*, *B. edematis maligni*, *B. putrificus* (Bienstock), etc., which also produce small amounts of butyric acid (Schattenfroh and Grassberger (320)), by the inability of the latter to cause stormy fermentation of milk, by the readiness with which they form spores in all media, and by the foul odor which they produce.

Attempts at classifying butyric acid bacteria have been made by von Hibler (144), Hirschmann and Lindenthal (149), Achalme (5), von Klecki (413), Passini (259), Rodella (287), Bredemann (37), and Schattenfroh and Grassberger. Bredemann, and Schattenfroh and Grassberger have made the most extensive studies of the problem of classification. The former was interested in *B. amylobacter* and considered the non-motile butyric acid bacilli only incidentally.

Schattenfroh and Grassberger (416) make the basis of their classification a phenomenon which they call "denaturability." Denaturing is a process which, with the adaptation to artificial media, especially solid media containing sugar, leads to a considerable increase of the thickness of the bacilli, and to loss of motility and power of spore formation. According to these authors, the motile spore-forming butyric acid bacillus (*B. amylobacter*) can not be denatured into the non-motile asporogenic form, but the non-motile form could easily be transformed into the motile sporulating type. Bredemann (37) confirmed this statement. He found that

"the gas phlegmon bacillus and the non-motile butyric acid bacillus of Schattenfroh and Grassberger underwent the same change (*i.e.*, transformation into a 'richly sporulating actively motile bacillus') and became identical with *B. amylobacter*."

The process by which the transformation was brought about was one of selection. Cultures containing spores (Schattenfroh and Grassberger (321) used egg heated for one hour under thirty pounds' pressure, Bredemann (37) used potato to which chalk had been added) were heated to 80°C. for thirty minutes, and transfers made to new tubes of the same kind of medium. By repeating this process a motile sporulating strain was produced. Even the location of the spore was changed, becoming terminal where it had formerly been central. The bacilli at the same time became

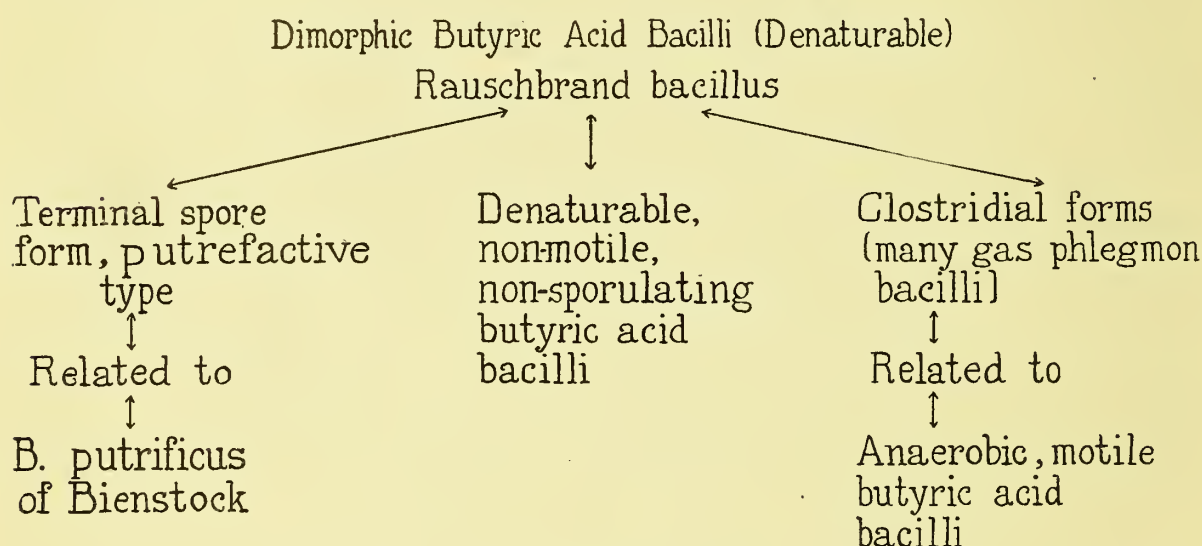


CHART 1.

thinner and resembled *B. putrificus*. These unusual results were, as already stated, confirmed by Bredemann.

Schattenfroh and Grassberger (321, 322) sum up their conclusions from their former work with the declaration that the so called Rauschbrand bacillus is a pure butyric acid bacillus which shows a double form, a sporulating, clostridial, motile, flagellated form which contains granules staining blue with iodine; and a non-motile, non-flagellated, non-sporulating form which does not contain iodine-staining granules. These two types differ further in the shape of the colonies on agar, in pathogenicity, in toxin formation, and in the amount of butyric acid produced. But they can be transformed one into the other, more easily from the non-motile, non-sporulating form to the motile, sporulating variety than in the reverse direction. Chart I, modified by Sittler (340) from the more complicated table

of Schattenfroh and Grassberger, presents the possible transformations that may take place.

My own work has not been sufficiently extensive to disprove the possibility of these rather remarkable transformations. They do not coincide with the generally accepted view of the fixity of bacterial species. Furthermore, Klein's earlier statements concerning the two types of *B. enteritidis sporogenes* were later corrected by him when he discovered that he had been all the while working with mixed cultures, one organism growing best under certain conditions, the other being favored by different conditions.

Passini (260), who appears to have accepted the theory of transformations of the non-motile into the motile butyric acid bacillus, made comparative studies of the agglutination of the asporogenic and the sporogenic forms of *B. welchii* and of *B. putrificus*. The sporogenic *B. welchii* serum agglutinated both forms of *B. welchii* and *B. putrificus*. *B. putrificus* immune serum agglutinated the sporogenic form of *B. welchii*. Asporogenic *B. welchii* serum agglutinated the corresponding form but not the sporogenic variety or *B. putrificus*. These results make one suspect that the cultures which were so readily transformed into a bacillus like *B. putrificus* were not pure cultures.

By growing some of my cultures from a week to ten days in sugar-free broth containing a bit of coagulated egg-white under oil, heating the culture, and transplanting to fresh media of the same kind, it was possible in the course of six or eight such transplants to obtain a strain which produced spores somewhat earlier and more abundantly than at first. But it was never possible to detect any motility nor to produce sporulation in milk or other media containing fermentable sugars.

So much for the relation of the *B. welchii* group to related groups of anaerobic bacteria. It is evident from a comparison of the original descriptions of the six organisms mentioned above as probably identical and the descriptions of other organisms isolated by later investigators and identified with one or another of these, that the names *B. welchii*, *B. aerogenes capsulatus*, etc., represent a group and not a single strain. This is the opinion of Silberschmidt (337), Herter (132), Klotz and Holman (192) and others. Table I shows the characteristics of the six organisms as originally described.

In addition to these six organisms and others which have been more or less loosely identified with one or another of them, a number of strains have been isolated which probably belong to the group but show some more marked variation from the classical description than is usual. Buday (42) isolated from a case of postmortem subcutaneous emphysema a bacillus

TABLE 1.

Characteristics of Six Anaerobic Organisms, Probably Identical with or Closely Related to *Bacillus welchii*.

<i>B. aerogenes capsulatus</i> .*	<i>B. enteritidis sporogenes</i> **	<i>B. perfringens</i> ***	<i>B. saccharobutylicus immobilis</i> .†	<i>B. phlegmonis emphysematosæ</i> .††	Bacillus of Achalmé.†††
3-6 μ in length	2.5-3.5 x 0.8-1.25 μ	Size of <i>B. anthracis</i> ; ends somewhat square	2-8 μ in length	Rods, size of <i>B. anthracis</i> ; long filaments in gelatin and in infected animal; in milk short	Short in media with sugar, longer in sugar-free media; in body, size of <i>B. anthracis</i> .
Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive	Gram-positive.
Non-motile	Some individuals motile	Non-motile	Non-motile	Non-motile	Very slightly motile; motility quickly lost on cooling or exposure to air.
Spores on Loeffler's blood serum	Spores oval; central on blood serum	No spores seen	Sporulation best in 0.1% starch agar, at least 5% alkaline; spores terminal	Spores, round or oval, terminal; "inconstant and exceptional" in sodium formate and plain agar and blood serum	Spores, large, ovoid, terminal; in serum and amniotic fluid and sometimes in broth.
Capsules in fluid from lesions only		Capsulated in pus from lesions; not in cultures		Stained smears from milk cultures showed pale zone around bacilli	Capsules in fluids from body, not in cultures.

Agar colonies, round; up to 3 mm. in diameter; white to brownish white in color	Agar colonies, surface gray, round, flat, margin thin, and slightly crenated	Agar colonies, surface, resemble streptococcus colonies; yellowish, granular, transparent edge regular or undulated and very delicate	Two types; colonies on agar, round and branching	Agar colonies, surface round or ellipsoidal, dark brown center, periphery a fine network of filaments	
Chains in peritoneal exudate	Few short chains	Sometimes in chains	Sometimes in chains	Few short chains in media other than milk	Zig-zag chains frequent.
	Agar stab, linear growth; much gas	Agar stab, deep colonies round or lenticular; fragmentation of medium		Agar stab; gas formation breaks up medium	Agar stab; fragmentation of medium by gas.
Gelatin slowly liquefied	Gelatin slowly liquefied	Gelatin not liquefied	Gelatin not liquefied	Gelatin sometimes liquefied, sometimes not	Gelatin growth slow; liquefaction in 2-3 wks. if heavily inoculated.
Milk, stormy fermentation	Milk, stormy fermentation		Milk, stormy fermentation	Milk, stormy fermentation	Milk, coagulation; curd full of gas holes.
	Blood serum, slow liquefaction			Blood serum; gas and foul odor	

* Welch, W. H., and Nuttall, G. H. F., *Bull. Johns Hopkins Hosp.*, 1892, iii, 81.
 ** *Annual Reports of the Medical Officer of the Local Government Board*, 1901-02, No. 31, 404.
 *** Veillon and Zuber, *Arch. d. méd. expér. et d'anat. path.*, 1898, x, 539.
 † Schattenfroth, A., and Grassberger, R., *München. med. Wchnschr.*, 1900, xlvii, 1077.
 †† Fraenkel, E., *Ztschr. f. Hyg. u. Infektionskrankh.*, 1902, xl, 73.
 ††† Achalmé, P., *Ann. de l'Inst. Pasteur*, 1897, xi, 845.

which differs from *B. welchii* only in the formation of long filaments and in the production in gelatin, but not in agar plates, of colonies with hair-like processes. Welch (389) mentions an "aerobic gas bacillus" isolated in his laboratory by Lanier. Uffenheimer (375) and Legros (206) have described similar organisms. The bacillus isolated by Albrecht (11) from Case 2 of his series attacked sugars much more slowly than other strains of *B. welchii*. Stokes and Stoner (348) found an organism in vaccine lymph which, when first isolated, did not attack lactose but later acquired that property. Hoseman (155) and Bloodgood (32) isolated strains of *B. welchii* from subcutaneous infections which had not produced gas in the subcutaneous tissues of the patient and did not produce it in subcutaneous lesions in guinea pigs. Loris-Mélikov (225) described what he called the "*variété acétique*" of *B. perfringens*, the name being given because it produced acetic acid. Riegler and Jacobson (279) isolated from the stool of a child a bacillus which resembled *B. welchii* except that it had flagella and was motile.

Attempts to classify the members of this group are made difficult because of the disconcerting variations shown by the same strain after growing on artificial media for several generations. This tendency was noted by Schattenfroh and Grassberger (318), von Freudenreich and Jensen (93), and by Gehrmann (100).

Many of my cultures showed the same troublesome tendency to variation which all but frustrates any attempt at definite classification within the group. It is certain that if there are subgroups within the larger group of non-motile butyric acid bacilli, no given strain can be accurately identified until it has grown for several generations on artificial media under the most exactly uniform conditions.

Andrewes (13) calls attention to the fact that the absence of sexual reproduction is one reason for the great variability among bacteria, and that binary fission may be a reason for not applying Weismann's principle of non-inheritance of acquired characteristics to bacteria. It is possible that in *B. welchii* we have a group of bacteria not yet fully differentiated, in which the group characteristics have not yet become definitely fixed. Different strains of *B. welchii* with their variations will stand in somewhat the same relation to the more stable, anaerobic tetanus bacillus, as the more variable *B. coli* group stands to the more fixed *B. typhosus*.

Furthermore, in the case of the obligate anaerobes we have to consider a factor of the environment which is of relatively small importance in the vital processes of aerobes and facultative anaerobes; namely, the amount of free oxygen present in the medium in which they are growing. Most

of the anaerobes have an optimum oxygen tension, just as they have an optimum temperature. Thus Chudiakow (411) found that *Bactridium butyricum* grew freely in five millimeters (0.13 per cent.) of oxygen, scantily in ten millimeters, and not at all in fifteen millimeters. *Clostridium butyricum* (Pragmowski) grew freely in ten millimeters of oxygen. *B. edematis maligni* and *B. tetani* grew in twenty millimeters, and *B. chauvei* in forty millimeters.

The influence of the presence of traces of free oxygen showed itself in my cultures on numerous occasions. Tubes of milk which had not been rendered sufficiently anaerobic showed either no growth or simple coagulation without any trace of gas formation. The number of involution forms also seemed to be related to the degree of anaerobiosis.

But the amount of oxygen left in the media is not the only factor which causes variations among the bacteria of this group. The reaction of the medium and other now unknown conditions will produce variations.

The chief difficulty in the way of subdivision of this group of bacteria is the finding of some characteristic which is sufficiently stable to be depended upon as a basis of classification.

Klein (184) and Fraenkel (91) recognized only one variety of the organisms isolated by them; namely, the pathogenic. The non-pathogenic strains which resembled *B. enteritidis sporogenes* were classed by Klein as *B. butyricus* of Botkin. Fraenkel insisted that his *B. phlegmonis emphysematosæ* was not identical with the non-motile butyric acid bacillus of Schattenfroh and Grassberger (316), chiefly on the ground that his organism was virulent for guinea pigs and the latter was not. Hitschmann and Lindenthal (149) state that the pathogenic properties of the bacilli of this group are variable and of slight value in differentiation. The latter investigators noted irregular variations in the growth of their strains, especially in sugar gelatin, but were unable either from the behavior in cultures or in the animal body to differentiate varieties. These conclusions were based upon a study of six strains isolated from cases of "gangrène foudroyante."

Passini (260), Werner (392), and Rocchi (285) attempted to establish varieties by means of agglutination tests, Rocchi applying also the complement fixation test. Werner immunized four rabbits with four different strains of this group including one of Fraenkel's original strains of *B. phlegmonis emphysematosæ*. He found that each serum agglutinated its corresponding organism in dilutions of from 1 to 200 up to 1 to 1,000, but did not cause agglutination of any other strain even in dilutions of 1 to 10, except in the case of the serum obtained after injecting with an organism isolated from milk. This serum agglutinated a similar bacillus isolated from the same sample of milk. Rocchi obtained similar results in which

immune serum of the *B. perfringens* from his own laboratory would not agglutinate a *B. perfringens* obtained from Jungano. Neither serum would bind complement in the presence of the heterologous organism.

I immunized four rabbits with four different strains and tested the agglutinating power of the serum against twenty-three strains of *B. welchii* isolated from a variety of sources. All the sera were of low titer (1 to 40 up to 1 to 80). The results were too irregular to furnish a basis of classification. Organisms which were agglutinated by the same serum only occasionally showed the same fermentation reactions.

Herter (132) thought "there is reason to believe that there are subvarieties of Welch's gas bacillus, subvarieties based mainly on differences respecting the difficulty of sporulation, upon pathogenic qualities, hemolytic properties, indol production, rapidity of gas formation in man, etc." He did not report results of an attempt to apply these factors as a basis of classification.

Rosenthal (295) differentiates two varieties of Achalme's bacillus "*variété rhumatismale*" and "*variété banale*," or *B. perfringens*. The former he sometimes designates the "anemobacillus of acute articular rheumatism." The "*variété rhumatismale*" does not produce a fetid odor in cultures, egg-white is not dissolved, nitrates are reduced, saccharose is inverted, it produces articular rheumatism in rabbits, and can be transformed into the enterococcus. The "*variété banale*" produces a fetid odor in cultures, dissolves coagulated egg-white, uses saccharose without inversion, does not reduce nitrates, is non-pathogenic for rabbits, and can not be transformed into the enterococcus.

Jackson (164) studied two strains of *B. welchii*, one obtained from Professor Welch, the other from Professor Ernst, of the Harvard Medical School. He depended upon the fermentation of sugars as the chief basis of his classification. The differences he noted were as follows: Type A, received from Dr. Welch, was non-motile; produced 26 per cent. gas in raffinose broth which remained neutral in reaction; produced only 10 per cent. gas in mannite broth, the reaction remaining neutral; and produced 92 per cent. gas in lactose bile broth. Type B, obtained from Professor Ernst, was motile; produced 22 per cent. gas in raffinose broth, the reaction becoming acid; 56 per cent. gas in mannite broth, the reaction being neutral; and produced no gas in lactose bile broth.

In view of the confusion which exists in regard to classification within this group, a study was undertaken of a number of strains, isolated from various sources, with the hope of discovering some basis of classification which would permit a correlation of morphological and cultural character-

istics with pathogenic powers. Altogether about fifty strains were studied more or less thoroughly. Some of the cultures died out before a complete study could be made. Full records were obtained of some thirty organisms from the following sources: from human stools, 19; normal stools of adults, 2; diarrheal stools of adults, 1; normal stools of infants, 5; diarrheal stools of infants, 4; stools of patients with pernicious anemia, 5; stools of patients with typhoid fever, 2; from the soil, 2; from sewage, 1; from milk, 1; from bird feces, 1; from cow feces, 3; from the lumen of a normal appendix obtained at autopsy, 1; from the washings from vegetables (potatoes and lettuce) bought in the open market (Boston), 2.

The methods of isolation and the care used to obtain pure cultures are discussed in the section entitled "Method of Isolation." After isolation in pure cultures, the morphology and cultural characteristics in various media, special attention being given to sporulation, the fermentative powers, and the pathogenic and hemolyzing properties of each strain were studied. The following media were made use of: plain, dextrose, lactose, and starch agar; plain agar with coagulated egg-white; plain and sugar-free broth with and without coagulated egg-white; physiological salt solution with coagulated egg-white; sugar-free broth with fresh guinea pig spleen; sterile milk; Loeffler's blood serum; plain and dextrose gelatin; suspension of feces; Rettger's egg-meat mixture (276); and the following fermentable substances, dextrose, levulose, and galactose; lactose, maltose, and saccharose; dextrin, inulin, and starch; and glycerin and mannite.

The first difficulty met with was the variation from time to time in the action of individual strains, especially in the fermentation tests and less frequently in sporulation. Thus the amount of gas produced by the same strain from the same sugar was by no means constant. This was equally true of all sugars. The formation of some gas from a substance capable of fermentation by the strain in question was, however, constant. In the case of glycerin and inulin, on the other hand, there at first appeared to be some variation. Thus an organism which would not attack these substances one week in one lot of broth might appear to ferment them vigorously the next in a different lot of broth. This necessitated the repetition of the fermentation tests with these two substances from two to six times with each strain. All members of the group are able to produce more or less gas from sugar-free broth. This gas formation is accompanied by very slight increase in acidity of the medium. It was found by titrating the culture that the variation noted in the gas production in broth containing inulin and glycerin was not due to a variation in fermentation, but to a difference in the degree to which the broth itself was attacked. In

those tubes which showed gas formation without increase of acidity it was considered that the organism had not attacked the glycerin or inulin, especially if spores were found to be present. All the strains studied by me formed spores in mannite broth.

From the reactions mentioned above, it is suggested that the group of *B. welchii* may be subdivided into four subgroups on the basis of their ability to ferment glycerin and inulin with the production of acid and gas, or to form spores in media alkaline to litmus, containing these substances.

Subgroup I ferments both inulin and glycerin with the production of gas and increase of acidity. It does not form spores in media containing either substance. The members of this subgroup produce strong hemolysins and are quite pathogenic for guinea pigs.

Subgroup II ferments, with gas production and increase of acidity, glycerin but not inulin. It forms spores in inulin broth but not in glycerin broth. Most strains are hemolytic, a smaller proportion pathogenic.

Subgroup III ferments, with gas production and increase of acidity, inulin but not glycerin. It forms spores in glycerin broth but not in inulin broth. Hemolysis and pathogenicity were not fully tested, but appear to be variable.

Subgroup IV ferments neither glycerin nor inulin, and produces spores in both glycerin and inulin broth.

Table II shows the results of a study of twenty strains. While the ability to ferment glycerin and inulin appears to furnish a reasonable basis of classification, it does not differentiate pathogenic from non-pathogenic strains nor hemolyzers from non-hemolyzers. I have not been able to discover any morphological or cultural characteristics which will differentiate pathogenic from non-pathogenic strains. Even agglutination tests, as far as I have been able to carry them out, will not make such a differentiation. The natural tendency among bacteria seems to be toward loss of virulence, unless there is opportunity for its exercise. Pathogenic properties are lost much more quickly than cultural or morphological characteristics. If "passage through an animal increases the virulence of a strain because only the virulent ones which are left in the culture are able to survive, and these transmit that characteristic to their descendants," it would seem that the same principle of natural selection would operate when the same strain of bacteria find themselves confronted with the necessity of continued existence outside the animal body. Those best able to live on dead organic matter, presumably the less virulent, would survive. It is not unlikely, therefore, that it will prove as impossible to distinguish the

TABLE II.
Characteristics of Twenty Strains of Bacillus welchii.

Subgroup.	Cul- ture.	Fermentation.										Hemolysins.	Pathogenicity.	Spores.					Gelat in liquefied at 22° C.	Gelat in liquefied at 37° C.	Source of culture.		
		Dextrin.	Dextrose.	Galactose.	Glycerin.	Inulin.	Lactose.	Levulose.	Maltose.	Mannite.	Saccharose.			Starch.	Mannite broth.	Inulin broth.	Glycerin broth.	Sugar-free broth.				Broth and egg-white.	
I	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Normal stool (adult).
	19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Diarrheal stool (infant).
	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Normal stool (3 day old infant).
	22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Diarrheal stool (adult).
II	2b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Soil (Brenham, Texas).
	21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Normal stool (6 day old infant).
	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Cow feces.
	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Cow feces.
	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Cow feces.
	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Milk.
	32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult, pernicious anemia).
III	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult, typhoid).
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Normal appendix.
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Same as 1 (adult) after 6 mos.
	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Diarrheal stool (infant).
	30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult, pernicious anemia).
IV	3a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Boston street dust.
	23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult pernicious anemia).
	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult, pernicious anemia).
	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Stool (adult, pernicious anemia).

Under "Fermentation," + signifies the production of acid and gas; — indicates very slight or no increase in acid, usually with a small amount of gas.

pathogenic gas bacillus from the non-pathogenic by cultural and morphological tests as it is to differentiate virulent and avirulent typhoid bacilli by the same methods.

DISTRIBUTION.

Bacteria of the *B. welchii* group are extensively distributed in nature. They have been isolated frequently from dust (Albrecht (11)), from dust of operating room (Walker (389), Hewlett (138), Savage (312), Wild (396), and others). Herter (135) spoke of *B. welchii* as "so common an inhabitant of the air."

Soil.

The spores of organisms of this group are very numerous in most soils. They have been found in garden earth (Schattenfroh and Grassberger (323), McCampbell (407), Savchenko (314), Hitschmann and Lindenthal (149), Westenhoeffer (393) and Klein (185)); in dirt from the street (Klein (185), Hewlett (138), Wild (396)); in "strand mud" (Hewlett (140)); in dirt from coal mines (Klotz and Holman (192)). I have isolated and studied organisms of this group from street dirt in Boston and in Galveston, from dirt from a yard, from grains of oats and corn picked up in the street, and from dirt from the laboratory floor.

Houston (157) made a special study of the fate of spores of *B. enteritidis sporogenes* in soil. He inoculated with sewage soil which already contained spores of *B. enteritidis sporogenes* and studied the fate of sewage bacteria; namely, *B. coli*, streptococci, and *B. enteritidis sporogenes*. There was no diminution in the number of spores of *B. enteritidis sporogenes* during the first ninety days of the observations, and only a partial disappearance of them after a year. He found spores of this organism "very sparse" in virgin soils. In cultivated and polluted soils their number sometimes reached 10,000 per gram (156). Klein (185) found the spores of *B. enteritidis sporogenes* abundant in soil from a garden to which manure had been applied from four to six months before. The presence of considerable numbers of spores of organisms of this group may therefore be considered to indicate pollution of the soil either from human or animal sources, but their presence gives no indication of the time which has elapsed since the pollution took place.

Sewage.

Spores of this group of bacteria are found constantly in sewage (Klein (185), Klein and Houston (191), Houston (157), and Gehrman (100)).

B. aerogenes capsulatus was isolated from the contents of a cesspool by Harris (389). Klein (185) found from 200 to 2,000 spores of *B. enteritidis sporogenes* per cubic centimeter of raw London sewage. He further found the spores (not more than fifty per cubic centimeter) in all sewage effluents, whether after simple sedimentation, precipitation, or filtration. It is not clear whether any of the effluents tested were from intermittent sand filters. Klein and Houston (191) found spores of *B. enteritidis sporogenes* more abundant in "trade sewage" than in "purely domestic sewage."

Water.

Members of this group have frequently been isolated from water: from spring water by Schattenfroh and Grassberger (318); from river water (Chicago River by Gehrmann (100), Thames River by Klein (185) and by Houston (159), and Klein and Houston (190, 191)*; from well water by Houston (158). Henseval (472) found spores of *B. enteritidis sporogenes* in water from the Belgian "Küste." I have found *B. welchii* in two cubic centimeters of the water of Galveston Bay which receives the sewage from the city, as well as from the ships at the docks. Water taken from the Gulf of Mexico on the south side of Galveston Island, several miles from the outlet of the channel and a half mile from the bath houses, gave negative results with twenty cubic centimeter quantities. Houston (159) found no spores in uncontaminated sea water in 100 cubic centimeter amounts.

The significance of the presence of spores of *B. welchii* in water has received much attention. Klein and Houston (190, 191) believe that the presence of the spores of *B. enteritidis sporogenes* in conjunction with *B. coli* in water represents recent pollution with sewage. Houston (159) found from 1 to 100,000 (?) spores of *B. enteritidis sporogenes* per cubic centimeter of water from the Thames. Near the source of pollution *B. coli* outnumbered the spores of *B. enteritidis sporogenes*, while further down stream the proportion was reversed.

The last statement would appear to hold good for turbid waters only. Clear water, if polluted, is more likely to contain *B. coli* than the spores of *B. welchii*. Gehrmann (100) found spores of *B. enteritidis sporogenes* in only two samples of water from a Chicago tap at a time when 20 to 25 per cent. of the samples from the same source showed *B. coli*. Thresh (373) "frequently examined waters showing *B. coli communis* in one cubic centimeter in which the spores of *B. enteritidis sporogenes* could not be detected in 500 c.c." Boyce (409) does not regard *B. enteritidis sporogenes* as so reliable an indicator of sewage pollution as *B. coli*. The explanation for this discrepancy, according to Houston, is that the "spores, not being

motile, may be more easily arrested by the soil through which the water passes, and may have a tendency to attach themselves to small particles of insoluble matter floating in the water and be carried down therewith." The absence of spores of this group from clear waters known to be polluted may be due to a "filtration" (sedimentation) which removes the non-motile spores but is too imperfect to remove motile organisms like *B. coli* and *B. typhosus*.

Thresh (373), however, "lays considerable stress upon the milk test for the *B. enteritidis sporogenes* in water." He applies it as follows: 500 cubic centimeters of the sample of water are filtered through a porcelain filter tube. The material held back by the filter is washed off and suspended in a small amount of sterile water. One-fourth of this suspension is placed in one tube of recently boiled milk and the remainder in another. These tubes of milk are then heated to 80° C. for fifteen minutes, cooled, and incubated.

Hewlett (140) also considers the presence of *B. enteritidis sporogenes* in water as evidence of sewage pollution. To test water for the presence of these spores Hewlett used large tubes in which were forty cubic centimeters of sterile milk. To each of twelve such tubes he added sixty cubic centimeters of the water to be examined, and heated to 80°C. for fifteen minutes. This method permits the examination of 720 cubic centimeters of water. The milk diluted to the extent used still gave a typical stormy fermentation test with *B. enteritidis sporogenes*.

Stewart (346), on the other hand, believes that because of its wide distribution it has little value as an indication of sewage pollution.

Hachtel and Freas (408) found that in turbid waters which had given a test for *B. coli* in one cubic centimeter quantities, 0.75 to 1.0 part of available chlorine per million so reduced the number of *B. coli* that that organism could not be isolated from plates made from lactose-bile tubes inoculated with ten and fifty cubic centimeters of the water, respectively. These tubes showed gas and in all instances save one *B. welchii* or *B. sporogenes* were isolated from them. This fact places less value upon the presumptive tests for *B. coli* in waters treated with calcium hypochlorite.

Fish.

Herdman and Boyce (129) frequently found spores of *B. enteritidis sporogenes* in various shellfish bought in the general market. Houston (159) estimated that 8 per cent. of the oysters from one source contained 1,000 spores of *B. enteritidis sporogenes* per oyster. 44 per cent. of the oysters from the same source showed 10,000 *B. coli* per oyster. Stewart

examined sixty-three samples of shellfish of all kinds and found *B. enteritidis sporogenes* in seventeen. The Standard Methods for the Examination of Shellfish adopted by the Laboratory Section of the American Public Health Association make no provision for the testing for spores of *B. welchii*.

Stewart (346) examined fifteen specimens of salted fish (codfish, haddock, and ling) and found spores of *B. enteritidis sporogenes* in five. In this connection it is interesting to note that Peruansky (268) examined the intestinal contents of a number of fresh water fish (*Perca fluviatilis* and *Barba fluviatilis*) and found only two anaerobes, neither of which resembled *B. welchii*.

Milk.

Flügge (87) in 1894 isolated from milk anaerobic butyric acid bacilli whose spores were killed by boiling for one hour. In the same year Botkin (35) described an avirulent organism which he isolated from milk and designated as *B. butyricus*. His technique was not above criticism, and it seems probable, as Schattenfroh and Grassberger (316, 317) pointed out, that his cultures contained the non-motile butyric acid bacillus contaminated with a motile organism.

Klein (181) and Andrewes (10) isolated *B. enteritidis sporogenes* from the milk used in St. Bartholomew's Hospital on the days on which epidemics of diarrhea occurred, as well as from "most samples" of market milk examined (185). Hewlett (138) found the spores in eight out of fifteen samples of milk tested. Gehrman (100) isolated *B. enteritidis sporogenes* from samples of milk sold in Chicago, but all the strains proved to be non-virulent. Stewart (346) found spores of that organism in 49 out of 213 samples of milk, and of these 38 were pathogenic. Brown (40) isolated from milk strains of *B. aerogenes capsulatus* which, when grown in sugar-free broth plus tissue, were pathogenic for guinea pigs. These same strains, when grown in plain broth and tissue, produced only a subcutaneous nodule when injected into guinea pigs.

Schattenfroh and Grassberger (318, 320) isolated their non-motile butyric acid bacillus from 80 per cent. of the samples of market milk in Vienna. Jacqué (165) found the same organism in a similar percentage of milk.

Dold and Garrett (68) failed to find spores of members of this group in samples of condensed milk examined. Andrewes (471), however, appears to have found these spores in condensed milk. Tissier and Gasching (367) do not mention *B. welchii* among the bacteria taking part in the fermentation of milk.

I found the organism present in 90 to 95 per cent. of the samples of Galveston milk examined. One strain isolated from that source showed an unusually high degree of virulence for guinea pigs.

Savage (312) considers the test for *B. enteritidis sporogenes* of especial value in determining the degree of cleanliness with which the milk was obtained and handled. He stated that there was no evidence of multiplication of this organism in milk kept at 15° to 18°C. This conclusion was based upon the examinations of the same sample of milk at intervals for spores of the bacillus. He used twenty cubic centimeters of milk distributed in two cubic centimeter quantities in ten tubes heated to 80°C. for fifteen minutes, and incubated in a Buchner or Novy jar. Of eighteen samples of milk kept at 20° to 21°C. for eighteen to twenty-four hours, nine (50 per cent.) showed no change in the number of spores of *B. welchii*; six (33 per cent.) showed an increase (one or two in twenty cubic centimeters); while three (17 per cent.) showed a decrease (one or two per twenty cubic centimeters). Savage, therefore, considers *B. welchii* a non-multiplying organism in market milk and therefore a good index to cleanliness at the farm (313).

In drawing that conclusion Savage overlooks one very important biologic fact with reference to this group of bacteria; namely, that they do not form spores in milk. If there were multiplication of *B. enteritidis sporogenes* in the milk it would not show itself in an increase in the number of spores. The multiplication would more probably be indicated by a diminution in the number of spores caused by the germination of some of those originally present.

In a few experiments carried out by me this actually appears to take place. On two occasions I found positive tests in three and four tubes out of ten of fresh milk and in only two tubes of each lot from the same milk which had stood for eight hours at room temperature. These tests were made with milk which contained many spores of liquefying bacteria and the tests were not always satisfactory. But they are at least suggestive that a diminution in the number of spores in milk may be associated with an increase in the actual number of *B. welchii* present.

Savage considers milk which shows a positive test in one or none of the ten tubes as "good;" milk which gives positive test in two or three tubes as "unsatisfactory;" and milk which shows stormy fermentation in five or more tubes as "bad." This is probably a fair statement. In my own rather limited experience it has proved an almost invariable rule that those samples of milk which showed large numbers of the spores of *B. welchii* also showed visible dirt or other indications of lack of cleanliness in obtaining and handling the milk.

Barthel (20) found only small numbers of anaerobic bacteria in ordinary milk. When present there were, almost without exception, either *B. putrificus* or the non-motile butyric acid bacillus. The number of anaerobes present was greater in summer than in winter. In autumn and winter butyric acid bacilli (*B. welchii*) were more numerous than *B. putrificus*. In spring and summer the latter organism predominated. Barthel did not believe that there was any relationship between the hygienic conditions under which the milk was obtained and the occurrence of obligate anaerobes. Of eighty samples of milk examined by him thirty-seven contained *B. welchii*.

Vincent (402) cites *B. putrificus* and *B. welchii* as examples of bacteria which grow in pasteurized milk. "Their products are of a highly irritant and dangerous character." In raw milk *B. welchii* never develops, but in milk heated to 170°F., *B. putrificus* usually, and *B. welchii* sometimes, will be present in nearly pure culture.

Cheese.

Schattenfroh and Grassberger (318) found their non-motile butyric acid bacillus in cheese. They did not consider that it had anything to do with the ripening of the cheese. Rodella (286) isolated the same organism from "hard cheese." Stewart (346), on the other hand, examined three samples of cheese for *B. enteritidis sporogenes* with negative results. Von Klecki (413) found a similar organism in hard cheese.

Miscellaneous Foodstuffs.

Spores of members of this group have been found in the widest variety of ordinary foodstuffs: in rice pudding made from milk which also contained the spores (Andrewes (10)); in sausage and in "dusty" sugar (Glynn (107)); and in wheat, rye, and barley flour (Schattenfroh and Grassberger (318)). Stewart (346) has made the most extensive search for spores of *B. welchii* in nature and has found them on wheat, oats, barley, maize, rice, lentils, haricot beans, and dried peas; in bran, oatmeal, malt, and flour; in canned sausage, margarine, and "tinned fruits." It is interesting to note that he failed to find the spores in any one of eleven samples of butter. Examinations on several occasions of the butter used in the Students' Boarding Club in Galveston have always given negative results in spite of the fact that from 90 to 95 per cent. of the samples of milk sold in the city showed spores of *B. welchii* in ten cubic centimeter quantities.

Conradi (53) examined bacteriologically the organs of freshly slaughtered cattle and hogs under careful aseptic technique. Of 162 organs exam-

ined, 72 contained bacteria, of which 30 were anaerobes, "the majority" belonging to the "butyric acid group." Tissier and Martally (366) found *B. perfringens* commonly present in the early stages of the process of putrefaction of meat, but it usually disappeared early.

I have isolated typical *B. welchii* from lettuce and potatoes bought of a Boston grocer.

Miscellaneous.

Dolley (69) found *B. aerogenes capsulatus* in 66 out of 250 blank cartridges examined. One brand showed 50 per cent. of positives. The powder in the cartridges was never found to contain the spores.

Stokes and Stoner (348) isolated a somewhat atypical strain of *B. welchii* from a specimen of vaccine lymph.

Welch (389) found *B. aerogenes capsulatus* on a bullet removed from the head of the tibia in a case of gas phlegmon; and Fraenkel isolated the same organism from a splinter of wood removed from a wound in a case of tetanus.

Anderson (462) found an organism closely resembling *B. welchii* in samples of crotalus venom placed on the market to be sold for the treatment of epilepsy. White (442) reports a case of gas bacillus infection following a snake bite.

Animals.

B. welchii has been found in the gastro-intestinal tract or in the feces of all domestic animals and of a large number of wild animals especially after being kept in captivity. It has been discovered in the feces of both carnivora and herbivora; for example, in feces of dogs, even young ones (Korentchevsky (195), Welch (389), Herter (132)); of cats, lions, tigers, and wolves (Herter (132)); of a rat when fed on egg but not when fed on carrots (Metchnikoff and Wollman (240)); of the rabbit (Korentchevsky (195), Welch (389)); of the guinea pig (Albrecht (11)); of a marmot (Hopffe (154)); of the elephant, camel, and buffalo (Herter (132)); of the pig (Welch (389), Savage (312)); of the goat (Herter); of the sheep (Savage (312), Choukévitch (50)); of the horse (Choukévitch (49), Savage (312), Herter (132), Klein (185)); of the cow (Schattenfroh and Grassberger (318), Savage (312), Choukévitch (50)).

Welch (389) called attention to the frequency of blebs on the intestines of hogs which have been dead for some hours, and found that these were in most cases due to *B. welchii*. Von Hibler (147) isolated *B. enteritidis sporogenes* from the blood, liver, and spleen of a pig dead of swinepest. Klein (185) did not find *B. enteritidis sporogenes* in the feces of the hog.

Von Hibler (147) isolated *B. welchii* from the heart's blood of a cow dead of anthrax.

Choukévitch (50) claims to have found *B. welchii* in all specimens of cow feces examined, but does not mention tests for virulence. Wild (396), however, claims that the feces of the cow do not contain *B. enteritidis sporogenes*, but do contain *B. butyricus* (Botkin) which is non-pathogenic. Klein (185) also appears to adhere to that view. The statement of Savage (312) that *B. enteritidis sporogenes* is found in the feces of the cow seems to be based upon tests for pathogenicity. Neubauer (251) disagrees with all other observers in that he found very few anaerobic bacteria in either the large or small intestine of cattle. He describes four types, none of which resembled *B. welchii*. A case described by Hueter (417) is of interest in this connection. An injury to the upper part of the thigh and the lower half of Poupart's ligament, not healing readily, was treated with a "folk remedy," namely, fresh cow dung. This treatment was almost immediately followed by a rapidly spreading gaseous gangrene. No bacteriologic examination was made.

Peruansky (268) did not find *B. welchii* in the intestinal contents of fish (*Perca fluviatilis* and *Barba fluviatilis*).

B. welchii has been isolated by me from the following sources: horse manure, 3 specimens, all positive; cow manure, 11 specimens, 10 positive; feces of monkey, 16 specimens, 8 positive; bird feces (English sparrow), 2 specimens, both positive; feces of chicken, 2 specimens, both positive.

Human Body.

B. welchii has been isolated from various parts of the human body unassociated with any pathological condition. Bosc and Carrieu (34) found the bacillus of Achalme (*B. welchii*) on the skin of normal persons and of patients with rheumatism. They believe that the reported finding of this organism in the blood of patients with acute articular rheumatism is due to contamination from the skin.

Jeannin (418) and Sittler (340) found *B. welchii* in the mouths of newborn infants. Baumgartner (21) isolated it from mouths of adults, especially in connection with carious teeth. Gilbert and Lippmann discovered *B. welchii* in normal salivary glands and their ducts (419), and in the normal pancreas (420). It has been found in normal bile at autopsy by Gilbert and Lippmann (105), and by Williams (398) in twenty-six out of eighty gall bladders examined. It has been isolated from the normal appendix by Clopton (389), and by the writer from four normal appendices obtained at autopsy. Romanovitch (290) discovered it in an appendix which contained round worms and was somewhat inflamed.

B. welchii was found to have invaded the body after death in eleven of 106 autopsies by Howard (160). Strauch (352) isolated it from the heart's blood of two out of 2,000 bodies at autopsy. Lehmann (209) found this organism in the heart's blood in one out of ten persons dead of erysipelas. Warnekros (383) found it in the heart's blood of a patient dead of septic abortion. It has been isolated from the circulating blood by Lenhartz (211) in a case of puerperal sepsis; by Gwyn (118) in a case of chorea; and by Roger and Garnier (289), and by Rocchi (421) in experimental occlusion of the intestine. Garnier and Simon (426) found that invasion of the blood stream by the anaerobes from the intestine was not infrequent in those diseases associated with injury to the intestinal mucosa. In one case of typhoid fever they isolated *B. welchii* from the blood stream.

B. welchii has been isolated from the normal urine by Jungano (405), and from the normal vagina by Hallè, by Kronig and Menge (198), and by Scheidler (325). The last named author considers this organism a normal inhabitant of the vagina, a statement which is denied by Young and Rhea (446).

A discussion of the presence of *B. welchii* in the human intestine will be found under a separate head.

ISOLATION.

The method now universally used in detecting *B. welchii* in material suspected of containing it is that first described by Botkin (35). This consists in inoculating a tube of sterile milk with the material in question, heating to 80°C. for fifteen minutes, cooling, and incubating. A reaction to be considered positive must show (1) stormy fermentation, that is, coagulation, copious formation of gas which usually causes fragmentation of the curd and derangement of the cream layer; and (2) a detectable odor of butyric acid. That a milk culture so heated may contain gas bacilli and yet not show either of these characteristics has already been pointed out.

Other media have been utilized by various investigators. For example, Harrass (125) used a sterile suspension of chopped calf's brain or liver; Rodella (288) used a 7 per cent. butyric acid broth; Savchenko (314) used a mixture of milk and broth containing lactose and sodium lactate. But milk appears to be the best medium for the isolation of this organism. The spores are said not to grow well on solid media (346).

When the milk test has been found positive subcultures have been made by some authors directly from the milk without further attempt at isolation. This does not give pure cultures in all cases and probably accounts

for the confusing results reported by many investigators. Inoculations from the milk tube may be made into tubes of dextrose agar, plates poured and incubated under anaerobic conditions by any of the well known methods (Schattenfroh and Grassberger (318), Simonds and Kendall (338)). Finally, Veillon's method of inoculating a series of tubes of deep dextrose agar by transferring from one to another until a dilution is obtained which will give isolated colonies, has been extensively employed (Veillon and Zuber (380), de Gasperi and Savini (99), and others).

All the strains used in this study were isolated from their original sources by means of the heated milk test. Dilutions from the whey in tubes giving a positive result were made in series in cooled melted dextrose agar and poured either into "bottle plates" (338), or into ordinary Petri dishes. When the agar had thoroughly hardened in the latter the lids were removed and the plates placed in either an ordinary desiccator or a Novy jar. The dishes were separated by a glass rod, two millimeters in diameter, bent at an acute angle. Pyrogallic acid wrapped in dry filter paper had previously been placed in the bottom of the desiccator or underneath a "bridge" which supported the plates in the Novy jar. When the Petri dishes were in place a solution of sodium hydroxide was poured in and the top of the desiccator or jar quickly put in place. The air was then partially exhausted by means of a vacuum pump. The partial exhaustion of the air and the absorption of the oxygen by the alkali-pyrogallic acid mixture produced anaerobic conditions under reduced pressure until the production of gas by the fermentation of the dextrose made the pressure inside the jar more nearly equal to the atmospheric pressure outside. Milk cultures grown in such a partial vacuum exhibit a most violent type of fermentation. The bubbles of gas rise with the speed and abundance of bubbles of steam in a tube of vigorously boiling water.

After twenty-four to forty-eight hours' growth in the incubator characteristic colonies were fished into deep tubes of dextrose agar. After twenty-four hours' incubation stained smears were made from these cultures to determine their purity. If found to be pure the culture was kept for further study.

MORPHOLOGY.

B. welchii exhibits great variation in size and even in shape when grown on various media. In the tissues, tissue juices, and peritoneal exudate the organisms are very short and even coccoid (Hitschmann and Lindenthal (149), Jungano and Distaso (169), Runeberg (306)) and may resemble diplococci (Norris (254)). On artificial media they vary from 2 to 10

microns in length by 0.9 to 1.4 microns in thickness, and have rounded ends. As a rule, they are more slender in sugar gelatin and broth than in other media (Hitschmann and Lindenthal (149), Jungano and Distaso (169)). According to Runeberg (306) they are shorter in alkaline media than in neutral and acid media. Kamen (170), in his earliest subcultures, found slender Gram-negative and thicker Gram-positive "spinet-like" bacilli (contamination?). Stolz (349) claimed to have observed branching forms in old cultures. Passini (264) noted that the organisms in his sugar-free, trypsin-digest medium were slender; after five days' growth 2 per cent. of sugar was added and the bacilli became short and coccoid.

My own cultures showed the above mentioned tendency to be more slender in gelatin and broth. All of them showed a marked uniformity of size in media, both liquid and solid, which contained a fermentable substance and which, therefore, became acid in reaction; as, for example, broth plus potato, milk, and the different sugar media. In milk cultures the bacilli were usually short. In alkaline media and media containing no fermentable substance, there was great variation in length, ranging from organisms almost coccoid in shape up to long filaments.

The filaments were more numerous in liquid media containing coagulated egg-white. They also occurred occasionally in the glycerin broth cultures of those strains which did not ferment glycerin; and sometimes in inulin broth cultures of those strains which did not ferment inulin. These filaments varied in length from approximately fifteen microns up to threads whose length exceeded the diameter of the oil immersion field. They were never numerous in any culture.

Schattenfroh and Grassberger (318), Kamen (170), and Hitschmann and Lindenthal (149) found filaments more numerous in sugar gelatin. One of the strains isolated by Hitschmann and Lindenthal (149) was at first very short, but after passage through a guinea pig it grew in long filaments. Long thread-like organisms have been observed occasionally in the tissues and tissue juices by Hitschmann and Lindenthal (149), Levy (212), Wild (396), and Rist (280). Gwyn (118) observed filaments in cultures in fluid media several days old.

Schattenfroh and Grassberger (316, 317), and Grassberger and Passini (114) noted the presence of clostridia in cultures of the non-motile butyric acid bacilli. These forms are found in greater numbers in cultures of the motile butyric acid bacillus. This form is usually, but not always, accompanied by spore formation. These spindle-shaped organisms were noted in cultures of four strains of my series. Two of these strains were isolated from the soil, one from a normal appendix obtained at autopsy,

and one from the normal stool of a healthy infant six days old. The clostridia appeared as early as the second day in two cultures. They were never very numerous but were most abundant in cultures in broth to which coagulated egg-white had been added. One strain produced them in sugar-free broth and another in saccharose broth. Only two of these four strains belong to the same group as classified above. The whey from milk cultures and broth cultures of the organism which yielded clostridia most abundantly frequently had the consistency of mucus.

Involution Forms.

Involution forms have been noted by numerous observers. Dunham (71), Guillemot (115, 116), Rist (280), and Jungano and Distaso (169) found them on old cultures. Gwyn (118) saw irregularly staining, dentated, swollen, and club-shaped forms in smears from surface cultures. Schattenfroh and Grassberger (318) described crescents, clubs, and "closely packed discs." Kamen (170) found clubbed forms and spherical masses which resembled actinomyces in old dextrose gelatin cultures. Hoseman (155) saw drumstick-like bodies in agar cultures twenty days old. These were not spores and the organisms could not be transplanted. Fitzgerald (84), who has made the most extensive study of involution forms, noted their presence in sugar-free broth and in broth which contained raffinose, lactose, isodulcite, and amygdalin, and less frequently in broth containing mannite and inulin. Involution forms were less frequent in acid than in alkaline media. Arabinose media showed fewest of these forms. Fitzgerald (84) saw larger and more slender rods, clubs, vacuolated forms, threads, and coccobacilli. The last mentioned type was more numerous in amygdalin, inulin, raffinose, mannite, isodulcite, and arabinose media.

The involution forms most frequently met with in my cultures were crescent-shaped, club-shaped, large, thick, deeply staining, and large, thick, palely staining, and coccoid forms. These bizarre shapes appeared most frequently in media containing sugars. Although found in broth containing mannite which is not fermented by this organism, they were not observed in sugar-free broth, nor in broth or physiological salt solution with coagulated white of egg, if the medium was covered with a large layer of oil. They appear earlier (in twenty-four hours) and in greater numbers in cultures on dextrose agar slants. They were first observed in my series in cultures on agar slants grown according to Rickards's (432) method. In these cultures crescent-shaped organisms were so numerous that they were at first thought to be a contamination. Two were frequently found

united in such a way as to make a perfect circle. The next most frequent source of involution forms was the water forced to the surface by the gas produced in deep stabs or shake cultures in agar containing fermentable sugar. The degree of anaerobiosis appears, therefore, to be the chief factor in determining the presence of involution forms. In cultures grown under strict anaerobic conditions these forms are either very few in number or absent altogether until the cultures are more than ten days old. In cultures or parts of cultures grown under less strict anaerobic conditions the number and variety of involution forms are greatly increased.

Grouping.

Practically all the strains described in the literature grew singly, in pairs, or in short chains of three to six bacilli. Scheidler (325) did not note chain formation in the body. Welch (389) and Jungano and Distaso (169), on the other hand, found very long chains in the peritoneal exudate of experimental animals. Hitschmann and Lindenthal (149) mention the arrangement of bacilli in parallel rows or at right angles to each other.

In my cultures the grouping was frequently similar to that of *B. diphtheriæ*; that is, either a palisade arrangement or pairs of bacilli joined end to end at an obtuse angle. Chains were not numerous and most of them were short. A few were observed containing as many as twenty bacilli.

Staining.

The bacilli in young cultures in my series were all Gram-positive. The older the culture the greater the number of Gram-negative organisms to be seen in a smear. Inoculations from cultures in which all the organisms were Gram-negative invariably failed to grow. Decolorization by Gram's method, therefore, appears to indicate that the bacilli are dead. These results correspond to those obtained and reported by Hitschmann and Lindenthal (149), Rist (280), Jungano and Distaso (169), and Schattenfroh and Grassberger (318).

Schattenfroh and Grassberger (317) noted in their non-motile butyric acid bacilli (*i.e.*, *B. welchii*) that some of the organisms in smears from starch agar showed fine granules which stained a deep blue with iodine. These granules were more frequently seen in the motile butyric acid bacilli and were usually associated with spore formation. In extreme cases of such granulation even the spores showed the granules (114). Schattenfroh and Grassberger found these iodine-staining granules in a culture of Fraenkel's original strain of *B. phlegmonis emphysematosæ*, and von Hibler (147) observed them in a culture of *B. enteritidis sporogenes* ob-

tained from Professor Klein. The longer the period since the isolation of a given strain the less the tendency to show this granulation (416).

This phenomenon has not been noted by all observers. Lotti (227) failed to demonstrate it in the strains of the organism isolated by him. Not all my cultures were examined for these granules. Of about ten strains grown in both starch agar and starch broth none showed the iodine-staining granules. Passini (259) found that this phenomenon is of little value in detecting butyric acid bacilli in smears from the stool because other organisms, even *B. coli*, show the granulation in the presence of starch.

Motility.

The majority of observers agree with Welch and Nuttall (386) in stating that *B. welchii* is non-motile. *B. phlegmonis emphysematosæ* (Fraenkel (89)), *B. perfringens* (Veillon and Zuber (380)), and one type of butyric acid bacilli isolated by Schattenfroh and Grassberger (318) were all described as non-motile. Klein (184), as late as 1901-02, that is, after discovering the mixture of organisms in his earlier cultures, stated that some bacilli in cultures of *B. enteritidis sporogenes* were motile. The bacilli isolated by Achalme (1, 3) from cases of acute articular rheumatism were described as slightly motile, the motility being quickly lost on cooling or exposure to the air. The "short bacilli" in Achalme's culture were always non-motile. A somewhat atypical gas bacillus isolated by Stolz (349) was said to be motile and to possess peritrichic flagella. Schumacher (333), whose technique was not above criticism, described a culture of *B. welchii*, isolated from a lesion of the eye, as motile but without flagella. Rocchi's (284) specimens of *B. welchii* were non-motile, but he described in his cultures the giant or combined flagella ("*Riesen-oder zusammengesetzte Geisseln*") which were first observed by Neisser in cultures of *B. edematis maligni*.

None of the strains of *B. welchii* isolated by me showed motility in either acid or alkaline media. The technique employed in examining for motility was that described by Dunham (72); namely, the use of a properly bent capillary pipette sealed at one end and containing a mixture of solutions of sodium hydroxide and pyrogallic acid in the other.

Capsules.

As first pointed out by Welch and Nuttall (386), capsules have usually been demonstrated in suitably stained smears from the tissues of infected animals or in man. Levy (213) and Hitschmann and Lindenthal (149), however, were unable to find capsules in the tissues of animals infected with

strains isolated by them. Rist (281) observed them in smears of pus from cases of otitis media. In smears of pus containing this organism from the antrum, however, Lewis (215) found that the capsules were "not prominent." Capsules have not been observed on ordinary culture media. Gwyn (118) found them occasionally in smears from cultures on blood media, and Schattenfroh and Grassberger (416) in cultures in sterile beef muscle plus one or two drops of a 50 per cent. sugar solution. Herter (135) observed capsules in smears from stools.

My cultures showed capsules only in smears from animal tissues.

Sporulation.

There is marked variation among different strains of *B. welchii* in respect to the readiness with which they form spores. Numerous factors influence sporulation, among which reaction of the medium, presence or absence of fermentable sugar, and temperature are the most important. Jungano and Distaso (169) never found spores in media containing sugar, and Fitzgerald (84) noted the distinct inhibitory action of acid, whether added to the medium or formed by the fermentation of sugar. Jacqué (165) found that sporulation occurred only at incubator temperature, never in the ice chest nor at room temperature (22°C.). Bredemann (37) considers spore formation of little value in differentiation because those strains which have lost the power of producing spores can have the power restored.

Great irregularity in the size, shape, and location of the spores has also been observed. They are usually oval and large. Loris-Mélikov (223) describes them as among the largest spores known. In Fraenkel's (89) cultures the spores were terminal; in Schattenfroh and Grassberger's (416) and in Tissier's (361) either central or terminal; in Klein's (184) near the center; and in Gwyn's (118) usually central, occasionally terminal, causing little swelling of the body of the bacillus; in Dunham's (71), central and thicker than the bacillus. Muscatello (246) and Loris-Mélikov (223) agree that the spores are near one end, but never terminal.

Many strains isolated have not been observed to sporulate in the media used while under observation; for example, those studied by Levy (212), Hitschmann and Lindenthal (149), Rist (280), Guillemot (116), Pic and Lesieur (269), Spitta (343) and Lotti (227).

Sporulation has been obtained in a great variety of media. Fraenkel's (91) cultures after some years' growth on artificial media sporulated very inconstantly in sodium formate agar, neutral red agar, and on Loeffler's blood serum. Passini (263) succeeded in making one of Fraenkel's strains

form spores by growing it in symbiosis with *B. coli* on egg sterilized by heating in steam under a pressure of three atmospheres. Spores have been noted in sugar-free media by de Gasperi and Savini (99), and by Fitzgerald (84); and by Passini (262) in sugar-free trypsin digest in symbiosis with *B. coli*. They were produced on Loeffler's blood serum by Hewlett (138) (*B. enteritidis sporogenes* and Achalme's bacillus), d'Agata (8), Gwyn (118), Dunham (71), Klein (184), and Herter (132); and in broth serum by Muscatello and Gangitano (248). Herter (132) found spores in cultures grown on media containing blood. Klein (184) grew *B. enteritidis sporogenes* on urine-gelatin and produced spores. Spores were found in cultures grown in broth and physiological salt solution containing bits of coagulated egg-white by de Gasperi and Savini (99) and by Loris-Mélikov (223). Bredemann (37) used potato the surface of which had been rubbed with chalk to develop a sporulating race of the non-motile butyric acid bacilli. Hewlett (139) found spores in gelatin cultures of *B. enteritidis sporogenes* and of a bacillus originally isolated by Achalme.

Schattenfroh and Grassberger (317, 318) caused sporulation by growing in alkaline starch agar. The degree of alkalinity required varied with the organism, but 0.5 per cent. was the strength usually found best. Schattenfroh and Grassberger do not state what indicator they used in titrating their media. Jacqué (165), however, points out that the degree of alkalinity of 0.5 per cent. to phenolphthalein is efficient, but not of the same degree of alkalinity to litmus. If vigorous fermentation of the starch took place no spores were produced. The results of Schattenfroh and Grassberger have been confirmed by Albrecht (11), Runeberg (306), Stolz (350), and Jacqué (165). Schattenfroh and Grassberger (317) also succeeded in producing spores in alkaline starch broth, but not as readily as in the starch-agar.

Noguchi (253) and Fitzgerald (84) have searched for spores in media containing various sugars, glucosides, and alcohols. They found that sporulation occurred in cultures grown in sugar-free broth containing arabinose, raffinose, amygdalin, salicin, inulin, dulcitol, isodulcitol, mannitol, and sorbitol.

According to de Gasperi and Savini (99), *B. welchii* never sporulates in living tissue. When sporulation does occur it is in tissue that is physiologically dead. Von Hibler (146) found spores in the body only in the dry muscle in cases of slow infection. Hitschmann and Lindenthal (151), however, report finding spores of this organism in the tissue juices in two cases. Rist (281) produced spores in serous fluid from the tissues by sealing it in capillary tubes and keeping (in the incubator?) for several days.

Herter (132), Dunham (71), and Howard (160) found spores in the bodies of rabbits on which the Welch-Nuttall (386) test had been made.

All the strains isolated by me formed spores fairly readily in some one or another of the different media used, provided the cultures were grown in the incubator. The spores were large, thicker than the bacillus, and occupied either an eccentric or terminal position in the bacillus. In some instances the large oval spores were seen free in the field. The number of spores formed was never large. It was frequently impossible to demonstrate them in stained smears from cultures in which their existence could be proved by inoculating tubes of sterile milk with a few drops of the sediment from the bottom of liquid cultures or with a lump of solid medium taken from the bottom of the tube, heating the milk for fifteen minutes at 80°C. and incubating for twenty-four to seventy-two hours. In practically all my examinations for spores the heated milk test was used in preference to microscopic examination.

By this method the production of spores was demonstrated in the following media: By all strains in plain and sugar-free broth to which a bit of hard boiled egg-white had been added, in sugar-free broth alone, in mannite broth, in a sterile alkaline or neutral suspension of feces, and in plain agar in the bottom of tubes in which a bit of hard boiled egg-white had been placed. Deep tubes of plain agar containing small pieces of coagulated egg-white have proved to be the most useful medium for preserving stock cultures of *B. welchii*. They should be kept in the incubator for one week before being placed in the refrigerator. When growth occurred in physiological salt solution plus a piece of coagulated egg-white, spores were also produced in that medium. Three strains produced spores in plain gelatin grown at 37°C. for one week. Gelatin cultures of about one-half of my strains were examined for spore formation. A few organisms sporulated in this medium. Those strains which did not ferment glycerin produced spores in sugar-free glycerin broth; those which did not ferment inulin produced spores in sugar-free inulin broth. None of my cultures produced spores in Schattenfroh and Grassberger's alkaline starch agar (317). All of them fermented starch violently.

Growth in sterilized suspensions of feces or in broth, in symbiosis with *B. coli*, *B. subtilis*, or *B. prodigiosus* did not appear to affect in one way or another the power of sporulation of these organisms. Control tests showed that spores were produced in the same media by the same strain whether grown in symbiosis or not. Media in which sporulation did not occur in pure culture did not show it in symbiotic cultures. These results do not, therefore, sustain Sittler's (340) statement that growth in sym-

biosis with *B. coli* enables *B. welchii* to produce spores under conditions and in media in which it would otherwise be unable to sporulate.

The sporulation of *B. welchii* in suspensions of feces has an important bearing upon the relation of this organism to intestinal disturbances. A full discussion of the results obtained in this line will be reserved until the association of *B. welchii* with diarrhea is discussed. Here these results may be summarized briefly.

(1) Sporulation occurs promptly and abundantly in pure cultures in sterilized alkaline or neutral suspensions of feces, but not in sterilized suspensions that have been rendered 1 per cent. or more acid to phenolphthalein.

(2) The number of spores in unsterilized suspensions of feces kept in the incubator under anaerobic conditions for twenty-four to forty-eight hours shows a very pronounced increase.

(3) Spores in unsterilized suspensions of feces to which 1 per cent. dextrose, or lactose, or maltose has been added do not increase in number if the acidity reaches 4 per cent. or more to phenolphthalein, but may increase quite markedly if the acidity is 3 per cent. or less to phenolphthalein.

CULTURAL CHARACTERISTICS.

Members of the *B. welchii* group grow readily on most artificial media, best on those containing fermentable sugar. Stewart (346) is the only observer who reports difficulty in getting his cultures to grow on artificial, especially solid, media. Thorndike's (372) strains grew scantily when first isolated. De Gasperi and Savini (99) found *B. welchii* and *B. sporogenes* (Metchnikoff) exceptions to the general rule that anaerobes grow slowly on ordinary culture media. Runeberg (306) saw evidence of growth of *B. welchii* in broth in two hours, and production of gas in dextrose agar in four hours.

Relation to Oxygen.

B. welchii is a strict anaerobe. Gwyn (118), however, succeeded in getting a scant growth in the presence of oxygen in a medium composed of milk or broth and serous fluid or blood. Tarozzi (354) and Wrzosek (433) grew *B. welchii* aerobically in broth containing fresh sterile tissue, especially spleen and liver. Wrzosek (433) also succeeded in growing *B. welchii* in broth containing a small piece of sterile (raw?) potato.

Rosenthal (294, 295) claims to have brought about an aerobization of *B. perfringens*. The process occurred in four stages. In the first stage there was no loss of characteristic properties. In the second stage the

organism lost its fermentative and pathogenic powers but quickly regained them again when grown anaerobically. In the third stage these powers were permanently lost. In the final stage Rosenthal speculates that the microörganism will lose its morphology and acquire the aspect and characters of another bacterium. He claims to have seen *B. perfringens* in the third stage of aerobization transformed into enterococcus. He even appears to believe that the *Diplococcus rheumaticus* of Poynton and Paine is such a transformed *B. perfringens*. This work has not been confirmed.

Dextrose Agar.

In dextrose agar plates that are heavily seeded numerous gas bubbles are present after twenty-four hours' growth. In those plates which show no more than half a dozen colonies, as a rule, no gas is produced. Jungano and Distaso (169) consider the colonies on dextrose agar sufficiently characteristic for diagnosis. Bredemann (37), on the other hand, considers the morphology of the colonies on dextrose agar of little value in the differentiation of species. Werner (428) states that, grown for a long time on artificial media, *B. welchii* tends to lose its characteristic form of colony.

The surface colonies on this medium of all strains isolated by me were quite characteristic. In twenty-four to forty-eight hour plates they were round, one to two millimeters in diameter, grayish by reflected light, translucent or almost transparent by transmitted light. Under low magnification (No. 2 ocular and No. 3 objective) the colonies were opaque, yellowish brown in color, finely granular, and the edges had a frayed appearance, the individual bacilli, without definite arrangement with reference to each other, being distinctly visible. The deep colonies were not characteristic. The majority of them were lenticular, occasionally trilobate, the shape being determined, according to Orsós (255), by the "elastic resistance" of the medium and not by the nature of the organism. Herter (132), however, found that "on blood-agar plates many of the colonies appear as minute points which lie beneath the surface and develop into fuzzy spheres."

Deep stabs or deep shake cultures in tubes of dextrose agar are accompanied by abundant gas production which results in the fragmentation of the medium and the extrusion of turbid fluid on the surface. Although exposed to the air, this fluid contains living bacilli and growth may at first occur in the agar up to the very surface. This is due, as pointed out by Hitschmann and Lindenthal (149), to the anaerobic conditions produced at the surface by the rising of the hydrogen formed by the vigorous fermentation of the dextrose.

My results accord with those of Hitschmann and Lindenthal (149), Pic and Lesieur (269), and Norris (254), that growth is more vigorous in glycerin agar than in plain agar, especially in the case of those strains which ferment glycerin with the formation of acid and gas.

Gelatin.

Great variation in regard to liquefaction of gelatin has been noticed in different strains of *B. welchii*. The process of liquefaction appears to depend upon several factors. Lotti (227) found that it varied with the percentage of gelatin used. Bredemann (37), who used high percentage gelatin failed to observe liquefaction in cultures of strains which had been found by others to be liquefiers. Fraenkel (91) believed that it depended upon the cooking of the gelatin. Veillon and Zuber (380), and Jungano and Distaso (169) obtained liquefaction only in those tubes of gelatin which were heavily seeded. Runeberg (306) found very slow liquefaction at 22°C., but tubes of gelatin inoculated with *B. welchii* and incubated for five days at 37°C. would not afterwards solidify when placed in ice water.

Strains of *B. welchii* isolated by the following did not liquefy gelatin: Pic and Lesieur (269), Rist (280), Thorndike (372), Stolz (349), Werner (428), Stokes and Stoner (348), and Bredemann (37). The following report strains which slowly liquefied gelatin: Welch and Nuttall (386), Achalme (3, 5), Klein (184), Flexner (85), Schattenfroh and Grassberger (318), Hitschmann and Lindenthal (149), Guillemot (116), Gehrman (100), Hewlett (139), Rist (281), Fraenkel (91), Runeberg (306), McCampbell (407), and Lotti (227). Of two cultures isolated by d'Agata (8) from infections in patients injured in the earthquake in Sicily one liquefied gelatin, the other did not.

The organisms studied by me grew vigorously in dextrose gelatin, but, in most cases, very scantily or not at all in plain or sugar-free gelatin. Of the twenty-eight organisms of this series, six would not grow in plain or sugar-free gelatin. Of the twenty strains included in Table II, two did not grow in plain gelatin. Of the other eighteen organisms, eight were grown in plain gelatin at room temperature only, and of these only one caused definite but very slow liquefaction. Of the twelve which were grown at both 22°C. and 37°C., six did not liquefy, four liquefied at 37°C., but did not at 22°C., one caused softening at 22°C. but did not cause liquefaction at 37°C. One strain liquefied gelatin at both room and incubator temperatures. By liquefaction at incubator temperature is meant such a change in the gelatin that it will not afterwards solidify when placed

upon ice. In a number of the above-mentioned tests the growth was so scant at 22°C. that no just conclusions concerning the liquefying powers of the organism can be drawn.

Hewlett (139) and Lehmann and Neumann (434) state that members of this group liquefy Loeffler's blood serum. Runeberg (306) noticed that the consistency of the coagulated serum was changed, but there was no definite liquefaction. Gwyn (118), Dunham (71), Welch (389), and others found no liquefaction of this medium. Fraenkel (92) found that cultures of *B. phlegmonis emphysematosæ* on Loeffler's blood serum gave off a foul odor. None of my cultures were grown on Loeffler's serum.

Broth.

Growth in plain or sugar-free broth is never very abundant and manifests itself as a uniform clouding of the medium which clears by sedimentation in two to four days. *B. welchii* grows more readily in sugar-free media if one or two drops of a fermenting culture are used in inoculating it. Growth in these media, no matter from what culture the inoculation is made, is usually accompanied by the production of a small amount of gas. This production of gas from proteid media was first noted by Welch and Nuttall (386). It occurred with great regularity in my cultures. The amount produced rarely exceeded 5 per cent. and was not accompanied with any increase, or at most a very slight increase, in the acidity of the medium. Growth of my cultures in plain or sugar-free broth was accompanied by the production of an unpleasant, but not a putrid odor.

Growth of the organism isolated by Levy (213) gave to broth a slimy, stringy consistency. One of my cultures (No. 3 a in Table II) showed this characteristic constantly in broth. The whey in milk cultures of this organism was also stringy and mucus-like. This strain was isolated from the soil, fermented neither inulin nor glycerin, produced spores in broth, was hemolytic but not pathogenic, and differed in no other way from the other members of the subgroup to which it belongs.

The addition to the broth of a small piece of coagulated egg-white before sterilization causes a more abundant growth of *B. welchii*. Growth, much less vigorous, will occur in physiological salt solution containing coagulated egg-white. None of my cultures produced any visible change in the white of egg. There was no reduction in size and no blackening of the white mass. Tissier (365) and Distaso (63), however, found that coagulated egg-white was attacked very slowly. The latter found a minute amount of black deposit in tubes of this medium inoculated with *B. perfringens* (169).

Growth in peptone-free media does not occur. In one experiment 1 per cent. solutions of lactose in physiological salt solution and 10 per cent. gelatin solution, respectively, sterilized under oil, failed to show perceptible growth of *B. welchii* after ten days in the incubator.

Potato.

Growth on ordinary potato is usually not abundant (Fraenkel (91)). Norris (254) observed gas production and the odor of stale glue in potato cultures. Schattenfroh and Grassberger (318) mention softening of the potato immediately under the growing culture. None of my cultures were grown in ordinary potato tubes.

Following the technique of Wrzosek (433) and others, the different strains of this series were grown in tubes of broth containing pieces of potato and sterilized under oil. Growth was very vigorous. Gas production was abundant. In a few instances the piece of potato was penetrated by the bacilli, gas produced within it, and the mass made to float on the surface of the broth. No spores were produced in this medium.

Milk.

The growth of *B. welchii* in milk gives rise to its most characteristic reaction; namely, stormy fermentation. And yet the growth in this medium has been the source of no little confusion. Klein (181) first described a typical and an atypical reaction in milk inoculated with *B. enteritidis sporogenes*. The atypical reaction, coagulation with slow digestion of the curd and separation of the milk into a lower layer of coagulum and an upper layer of yellowish turbid whey, Klein (184) later found to be due to a contaminating organism which he named *B. cadaveris sporogenes*. Klein (181) also believed at first that the age of the milk had much to do with the occurrence of the reaction. He later discovered that old milk, if boiled for fifteen to twenty minutes immediately before inoculation, would give a typical reaction again. In Fraenkel's (89) earlier reports on *B. phlegmonis emphysematosæ* he stated that it did not change milk. Later he found that this organism caused stormy fermentation just as the other members of the group (91). Even in 1902 he wrote that *B. phlegmonis emphysematosæ* sometimes curdled milk without producing any gas (91). One of the organisms isolated by Lotti (227) caused a rapid precipitation of the casein without gas formation, without proteolysis, but with the production of a strong odor of butyric acid. Schattenfroh and Grassberger (318) also noted that their non-motile butyric acid bacillus sometimes produced no gas in milk, a phenomenon which they

thought due to a lack of a suitable degree of anaerobiosis in the medium. This last conclusion was borne out by my own results.

Each of fifty odd strains isolated by me caused stormy fermentation in milk provided the correct degree of anaerobiosis was obtained. When that optimum was not obtained there was either coagulation with little or no gas formation, or no growth at all, and, therefore, no change in the milk, depending apparently upon the amount of dissolved oxygen remaining in the medium. Not infrequently, after inoculating fresh material or pure cultures containing spores into milk and heating for fifteen minutes at 80°C. there was after incubation only coagulation with little or no gas formation. This occurred more frequently when milk was used, which had been sterilized and allowed to stand for a number of days, than when freshly sterilized milk was employed. The older milk contained more oxygen in solution than could be driven off by heating to a temperature below boiling. In none of these "no gas" tubes was there any digestion of the curd. Smears from these tubes showed pure cultures of *B. welchii* and other tubes of milk placed in boiling water for fifteen to twenty minutes, cooled, and then inoculated from such a "no gas" tube showed typical stormy fermentation after incubation. Hence failure to get a typical reaction after a heated milk test does not always mean the absence of spores of *B. welchii* from the original specimen.

FERMENTATION BY BACILLUS WELCHII.

B. welchii is a violent fermenter of sugars. The addition of a carbohydrate to any medium greatly enhances the growth of the organism in this medium. Indeed, Tissier and Martally (366) declare that *B. welchii* does not develop unless there is some sugar present at the beginning. In the case of my own cultures, it was difficult and sometimes impossible to obtain growth in sugar-free broth when inoculated from another culture in the same medium unless a large amount was used for inoculation. Inoculation from a fermenting culture into sugar-free medium or from a plain broth culture into plain broth resulted, as a rule, in copious growth.

The success that has attended the use of carbohydrates and other fermentable substances as a means of differentiation between members of the intestinal group of aerobes has led to an attempt to use these substances as a means of distinguishing members of the group of butyric acid bacilli. Schattenfroh and Grassberger think that the fermentation reactions of *B. welchii* are of no value as a basis of classification because of the great variation in the products of fermentation by the same organism in the same kind of medium at different times (316), and because there is no rule

by which it can be determined how much gas will be produced by the different strains (416).

Theobald Smith, on the other hand, considers fermentation reactions of great value in classification. He has made use of them in the study of anaerobes in general (438) and of *B. chauvei* in particular (439). Dr. Smith sometimes found it necessary to "freshen up" a culture by making two or three transfers to sugar media before recording important readings. He gave special attention to the following points: the sugars fermented, the amount of gas produced, and the gas formula $\frac{H}{CO_2}$.

Jackson (164) has also made use of fermentation tests as a basis of classification and has divided this group into *B. welchii* A and *B. welchii* B.

The annoying variation in the results of fermentation tests has already been mentioned under the discussion of classification. This variation is due to a variety of causes. Beijerinck (24) believed that the action upon carbohydrates varied with the degree of anaerobiosis. My own experiments prove that that is a very important, but not the only factor.

The organism isolated from vaccine virus by Stokes and Stoner (348) fermented lactose very slightly when first isolated, but later acquired the power of vigorously fermenting that sugar. Hitschmann and Lindenthal (149), on the other hand, noted a decreasing power of fermentation with the increase in the age of the culture.

Fraenkel (91) thought that the variation in gas production was due not to differences in the age of the culture but to variations in the medium. Kropáč (199) found that the presence of proteid was necessary to production of gas from sugars, a statement in harmony with my own experiments already mentioned. Schattenfroh and Grassberger (318) found that lactose was attacked more vigorously in milk than in broth.

Finally, if the cultures used are not pure, the contaminating organism will probably influence the amount of gas produced. For example, Herter and Ward (131) found that *B. welchii* produced more gas in sugar media when grown in symbiosis with *B. coli* than when grown alone. A very limited number of experiments of my own appeared to confirm that statement and to show that the same result is obtained when *B. welchii* is grown symbiotically with *B. subtilis*. In the latter instance the production of alkaline substances by *B. subtilis* probably neutralized some of the acid produced by *B. welchii* and the process of fermentation was therefore allowed to proceed for a longer time.

The number of organisms inoculated is probably also a factor in solid media and broth, but not so important, as will be shown later, when milk

is used as the medium. The results obtained by Hitschmann and Lindenthal (149) and by myself have already been mentioned; namely, that in thickly seeded dextrose agar plates abundant gas production occurs, while in plates showing only a few colonies no gas was produced. In deep dextrose agar tube cultures I have noticed failure in gas formation in tubes showing only two or three colonies in the deeper parts of the agar.

When sugar media are fermented gas is usually produced with astonishing rapidity. The reaction is usually brought to a standstill in less than forty-eight hours by the acid produced. However, R. M. Smith (341) makes the surprising statement that the gas bacillus "if given a carbohydrate diet will continue to multiply almost indefinitely since it forms a very small amount of acid and therefore its reproduction is not self-limited." In all the monosaccharides and disaccharides and in some of the polysaccharides the gas produced amounts to from 50 to 100 per cent.

Gas production by *B. welchii* in the following carbohydrates and alcohols has been reported: dextrose, lactose, and saccharose by practically all investigators; levulose (Schattenfroh and Grassberger (318), Bredemann (37)); galactose, maltose, and glycerin (Schattenfroh and Grassberger (318), Achalme (5), Bredemann (37)); starch (Schattenfroh and Grassberger (318), Tissier (362, 363), Wollman (400), Choukévitch (50)); and dextrin and inosite (Achalme (5)). No gas formation has been noted in the following: mannite (McCampbell (407), Achalme (5), Lotti (227), Bredemann (37)); inulin (Achalme (5), Lotti (227)); dulcitol, erythritol, and amygdalin (Achalme (5)); and cellulose (Wollman (400)). According to Achalme (5) bacilli of this group ferment maltose more vigorously than any other sugar. Morse (244) found that children suffering from so called gas bacillus diarrhea do worse on maltose than on lactose.

The products of fermentation of sugars by *B. welchii* are chiefly acids and gases. The following acids have been found as the products of the fermentation of sugars: butyric, lactic, valerianic, propionic, formic, and acetic acids. Acetic, formic, propionic, and valerianic acids have been found only in small amounts by nearly all observers. Loris-Mélikov (225), however, states that acetic acid was the chief product of fermentation by his "*variété acétique*" of *B. perfringens*, while formic acid was the principal product of the ordinary variety. The relative amounts of butyric and lactic acids in a culture vary greatly. Both are produced from all fermentable substances, especially from the monosaccharides, disaccharides, and starch. According to Schattenfroh and Grassberger (416), butyric acid is the chief product only in milk, lactic acid being produced in greater

abundance in sugar broth. They found that the proportion of butyric to lactic acid may vary from 1 to 5 to 1 to 12 (316). In some instances in milk cultures the odor of butyric acid is very faint after twenty-four hours' incubation, but becomes strong if left standing at room temperature (Orton (256)). No attempt was made in my cultures to identify the acids, except butyric acid, by the odor.

The total amount of acid produced shows considerable variation. Some of Runeberg's (306) cultures in dextrose broth required 6 c.c. of $\frac{N}{1}$ sodium hydrate solution to neutralize 100 cubic centimeters. Loris-Mélikov's (225) cultures showed an acidity equivalent to from 2.94 to 3.43 parts per thousand of sulphuric acid.

The total acidity, with phenolphthalein as indicator, produced by some of my cultures in the different sugars is shown in Table III.

TABLE III.

Total Acidity Produced in Seventy-two Hours by Representative Strains of Bacillus welchii in Broth (0.8 Acid to Phenolphthalein) Containing Various Sugars. Results Stated as the Number of Cubic Centimeters of $\frac{N}{1}$ Sodium Hydrate Solution Required to Neutralize 100 Cubic Centimeters of the Broth.

Organism No.	Dextrin.	Dextrose.	Glycerin.	Inulin.	Lactose	Levulose.	Maltose.	Mannite.	Saccharose.	Starch.
6			2.2	4.0						
9			1.8	2.4						
17			2.2	3.4						
20	2.0	2.2	1.8	1.8	3.0	3.4	2.2	1.0*	2.0	3.6
21	2.2	2.2	1.8	1.0*	3.0	3.2	2.2	0.8*	2.2	3.4
23			1.0*	1.0*						3.2

* Spores present in culture.

Kamen (170) found small amounts of ethyl alcohol in milk cultures. Schattenfroh and Grassberger (416) mention butyl alcohol as present in cultures of non-motile type.

The gas produced by the fermentation of sugars by *B. welchii* consists chiefly of hydrogen and carbon dioxide. The proportion of these gases with reference to each other appears to vary within rather narrow limits. The following gas formulæ have been found:

Dunham (71)	$\frac{H}{CO_2} = \frac{64.3}{27.8}$	Nitrogen 8.1 per cent.
Keyes and Gillespie (177)	$\frac{H}{CO_2} = 1.48$	
Herter (133)	$\frac{H}{CO_2} = \frac{3}{1} \text{ to } \frac{2}{1}$	
Smith (438) *	$\frac{H}{CO_2} = \frac{2}{1} \text{ to } \frac{3}{2}$	
Hitschmann and Lindenthal (149)	$\frac{H}{CO_2} = \frac{67.55}{30.62}$	
Stolz (349)	$\frac{H}{CO_2} = \frac{2}{1}$	

Gas analyses in my cultures are given in Table IV.

TABLE IV.

Gas Formulæ of Ten Strains of Bacillus welchii Grown in Media Containing Different Sugars.

Organism No.	Dextrin.	Dextrose.	Glycerin.	Inulin.	Lactose.	Levulose.	Maltose.	Mannite.	Saccharose.	Starch.
1†	2/1	3/1	**	3/1	3/2	2/1	3/2	0	2/1	2/1
2†	3/1	3/1	**	2/1	2/1	2/1	3/1	0	3/2	2/1
3†	3/1	4/3	**		2/1	2/1	2/1	0	2/1	5/3
5	2/1	2/1	**	**	3/1			0	3/2	3/2
A (o.s.*)		3/1			3/1		3/1		3/1	3/1
B (o.s.*)		3/1			3/1		3/1			
C (o.s.*)		3/1			4/1		2/1			
D (o.s.*)		4/1			4/1		3/1			
E (o.s.*)		4/1			3/1		3/1			
F (o.s.*)		3/1			2/1		2/1			

* Old Series; that is, a series of strains isolated in 1912, most of which were lost.
** From 5 to 20 per cent. of gas, none of which was absorbed by sodium hydrate solution.
† Nos. 1, 2, and 3 in a later experiment fermented glycerin and inulin with production of acid and gas. The cultures were used for titration, no absorption test being made upon them.

In the case of all the strains studied, alkalization of the whey of milk cultures and of lactose broth cultures resulted, after standing for from six to forty-eight hours, in the production of a brilliant cherry red color.

This phenomenon is not characteristic of *B. welchii*, however, inasmuch as I have noted it in cultures in milk and lactose broth of *B. botulinus*, *B. chauvei*, and *B. edematis maligni* obtained from Professor Winslow. This same phenomenon was also noted by Brown (40) in milk and lactose broth cultures of anaerobes isolated from market milk. The substance which causes this color has not yet been determined. It is not acetyl-methylcarbinol which Harden (476) believes to be the substance responsible for the Vosges-Proskauer reaction noted in dextrose broth cultures of *B. aerogenes* and a few other aerobes. The substance which produces the red color in lactose-containing cultures of *B. welchii* and other anaerobes is not volatile, is not destroyed by boiling for an hour, changes to yellow or orange on acidification, and regains its redness on realkalinization. It is soluble in water, but not in alcohol, ether, chloroform, acetone, or toluol. Professor W. C. Rose, who kindly made a few preliminary tests to determine the chemical nature of this substance, found that it was not precipitated with phosphotungstic acid nor with tannic acid. The filtrate from tannic acid mixture was colorless until shaken vigorously, when it regained its red color. The color faded again very quickly, due probably to the strong reducing power of the tannic acid. This indicates that the colored substance is an oxidation product of some colorless compound. It does not seem to be produced from any substance other than lactose. However, I have obtained a slight reddening of the medium in 5 per cent. dextrose broth cultures, but never in 1 per cent. dextrose broth.

ACTION ON PROTEIDS.

The relation of *B. welchii* to proteolysis has been the subject of no little confusion. Herter (132) speaks of the "strictly anaerobic putrefactive bacteria, such as *B. putrificus* and *B. aerogenes capsulatus*," and states that the latter "attacks proteids vigorously forming hydrogen, carbon dioxide, and perhaps methane gas." Metchnikoff (236) speaks of *B. welchii* as one of the putrefactive bacteria of the intestine. Hitschmann and Lindenthal (149) believed that *B. phlegmonis emphysematosæ* could split proteid with gas formation, but did not class it as a putrefactive organism. Loris-Mélikov (224) ranges the three important intestinal anaerobes in the order of the intensity of their putrefactive action upon meat, as follows: *B. putrificus*, *B. sporogenes*, Metchnikoff (236), and *B. perfringens* (*B. welchii*).

Rettger (276), on the other hand, declares that *B. welchii* is preëminently a fermentative organism, and attacks proteids only very slightly, and then only in the absence of carbohydrate from the medium. In egg-meat mixture he found that *B. welchii* produced an unpleasant odor,

but that this was not putrefactive in character and not due to mercaptan (277). Herter (135) stated that *B. welchii* produces ammonia in "nearly sugar-free media;" and that it could not attack "egg-meat mixture," but was nevertheless intensely putrefactive when grown in media containing a low percentage of sugar. Lotti (227) found that his strains of *B. welchii* attacked proteid only very slightly. Schattenfroh and Grassberger (319) did not believe *B. phlegmonis emphysematosæ* capable of attacking proteid, at least not sufficiently to class it as a putrefactive organism. They noted that in the tissues it selects glycogen which accounts for its predilection for the liver. Distaso (65) removed sections of the intestine at autopsy, preserved them in a moist chamber in the incubator and attempted to determine which of the intestinal bacteria took part in the putrefaction of the intestinal wall. He emphasized the fact that *B. welchii* had nothing to do with the process.

Tissier and Martally (366), on the contrary, believe that *B. perfringens* (*B. welchii*) plays an important part in putrefaction, especially in the earlier stages. They state that it is able to act upon carbohydrates, proteids, and fats, and that it was the only species isolated by them which produced a secretion similar to that of the pancreas; namely, with trypsin, amylopsin, and steapsin. It would appear, however, that the action of the strain of Tissier and Martally (366) upon proteid (fibrin) was very slow.

This evident confusion concerning the action of *B. welchii* upon proteids may have several explanations. In the first place, there is a variation among different strains of the same organism. Herter (132) quotes Theobald Smith, for instance, as stating that the production of indol is characteristic of some strains, and failure to produce it characteristic of others. Hewlett (141) also states that "indol may or may not be produced." A culture of *B. enteritidis sporogenes* isolated by Hewlett (138) liquefied Loeffler's serum much less vigorously than specimens of the same organism isolated by Klein. It is possible, however, that the cultures of the latter were contaminated with *B. cadaveris sporogenes*, a very rapid liquefier.

Schattenfroh and Grassberger (416) were the first to distinguish between the action of the sporulating and the non-sporulating forms of this organism. The former they considered strongly proteolytic and putrefactive, the latter not. Passini (264) reached the same conclusion. He was able to transform the non-sporulating form into the sporulating by growing it in symbiosis with *B. coli*. It would seem that this distinction is not altogether justified because of the impossibility of growing the two forms

on the same media. The products of growth of the two types are not, therefore, entirely comparable. Each of my twenty-eight strains when grown on sugar-free media formed spores, some more promptly and abundantly than others. None of them ever formed spores in acid media, even though sugar-free, or in media which contained fermentable carbohydrate. A sporulating culture inoculated into a medium containing sugar immediately ceased to sporulate and the spores present all germinated. A non-sporulating culture inoculated into a sugar-free medium would always form spores sooner or later and in greater or less abundance, unless enough of the medium from the non-sporulating culture was carried over to render that being inoculated too acid to permit the formation of spores. Hence the distinction between the actions of the sporulating and non-sporulating form upon proteid would not seem justifiable. The inability to grow the two forms on the same media renders it impossible to compare their actions correctly. Furthermore, the proteid substance used in the test may influence the result. Tissier (365) states that *B. perfringens* shows a predilection for vegetable proteid. Tissier and Martally (366) have arranged animal proteids in the order of the ease with which they are attacked by *B. perfringens*, as follows: egg-white, egg-yolk, milk (casein), flesh, and fibrin.

The reaction of the medium and the presence of fermentable carbohydrate are likewise important. Most investigators (Passini (264), Schattentfroth and Grassberger (318), and others) agree with Rettger (277) in the statement that *B. welchii* acts upon proteid only in the absence of sugar. Tissier (365), however, claims that *B. perfringens* (*B. welchii*) "has a feeble proteolytic power in the presence of albuminous matter alone, and has a much higher proteolytic power in the presence of carbohydrate." In the presence of the latter the vitality and digestive activity of the organism are increased. Its "activity is increased ten-fold in a mixture of albumin and starch." Tissier further states that *B. perfringens* digests three-fourths of the casein in milk before the acidity has developed to a point to put a stop to its growth. This is a direct contradiction to the findings of Kendall (437) with reference to aerobic bacteria; namely, that in the presence of carbohydrate, proteid is not attacked; and to the findings of Schattentfroth and Grassberger (318) who were unable to detect any evidence of digestion of the curd in milk.

A very limited number of not altogether satisfactory experiments (because of the scantiness of the growth in the sugar-free medium), carried out according to Dr. Kendall's technique and under his direction, indicates that *B. welchii* does not differ from aerobes in its attitude toward pro-

teids in the presence of sugar. The scantiness of the growth in the sugar-free media will not permit the conclusion that *B. welchii* will not attack proteid; but the absence of any increase in ammonia in the vigorously growing cultures in broth containing sugar is proof that the presence of a fermentable carbohydrate does not increase the proteolytic powers of all strains of this organism. These findings are exactly contrary to the statement of Tissier (365). The results are shown in Table V.

TABLE V.
The Proteolytic Powers of Bacillus welchii in Sugar-Free and Dextrose Broth.

Organism.	Age of culture.	Ammonia in sugar-free broth.			Ammonia in dextrose broth.			Total acidity in sugar-free broth.			Total acidity in dextrose broth.		
		Control (uninoculated).	Culture.**	Excess of ammonia in culture.	Control (uninoculated).	Culture.	Excess of ammonia in culture.	Control (uninoculated).	Culture.**	Excess of acidity in culture.	Control (uninoculated).	Culture.	Excess of acidity in culture.
No. 7 (o.s.)	24 hrs.	4.5	4.6	0.1		4.5	0.0						
			4.5	0.0	4.5	4.6	0.1	0.2			0.8		
	3 dys.	4.5	4.5	0.0		4.3			0.2	0		3.0	2.2
			4.5	0.0	4.4	4.4	0.0	0.2	0.4	0.2	0.8	3.0	2.2
	5 dys.		4.5	0.0		4.4	0.0		0.5	0.3		2.3	1.5
			4.6	0.1		4.4	0.0	0.2	0.4	0.2	0.8	2.3	1.5
	7 dys.	4.4	5.5*	1.1*		3.4	—		0.2	0		2.4	1.6
			4.4	0.1	4.5	3.3	—	0.2	0.3	0.1	0.8	2.2	1.4
	9 dys.		4.3	0.0		?						2.2	1.4
			6.3*	1.8*				0.2			0.8	2.4	1.6
	11 dys.	4.5	4.5	0.0		4.2	—						
			7.0*	2.5*	4.3	?		0.2			0.8		
No. 16 (o.s.)	24 hrs.	4.5	4.6	0.1		4.4	—						
			4.5	0.0	4.5	4.2	—	0.2			0.8		
	3 dys.	4.5	4.4			4.3			0.4	0.2		2.8	2.0
			4.4		4.4	4.5	0.1	0.2	0.4	0.2	0.8	2.7	1.9
	5 dys.		4.5	0.0		4.4	0.0		0.4	0.2		2.0	1.2
			4.3			4.3	—	0.2	1.0*	0.8	0.8	2.4	1.6
	7 dys.	4.4	4.4	0.0		4.3	—		0.4	0.2		2.0	1.2
			4.5	0.1	4.5	4.3	—	0.2	0.4	0.2	0.8	2.4	1.6
	9 dys.		4.3			4.3	—						
			4.4			4.3	—	0.2			0.8		
	11 dys.	4.5	4.5	0.0		4.4	0.1						
			6.2*	1.7*	4.3	4.5	0.2	0.2			0.8		

* Culture contaminated.
** Growth in sugar-free broth very scanty; in dextrose broth very vigorous.

A variety of ferments have been isolated from cultures of *B. welchii*. Passini (264) noted the presence of a proteolytic ferment. Martin (233) found proteolytic enzyme in the filtrate from liquefied Loeffler's serum, but not from cultures in liquid serum or "albumin broth." Tissier and Martally (366) claim that *B. perfringens* (*B. welchii*) produces tryptic, amylolytic, and steaptic ferments.

A great number of split products of proteid have been reported in cultures of *B. welchii*. Indol was found by Tissier (363, 365), Lotti (227), Metchnikoff (238), Passini (261) (in serum only), Herter (132) ("in suitable media"), and Loris-Mélikov (226). Negative results were obtained by Achalme (5), Jungano and Distaso (169), Runeberg (306), and Norris (254). None of my cultures produced indol in sugar-free broth. They grew too poorly in peptone solution to justify any conclusions from the failure to get a positive test for indol in the cultures in that medium.

Skatol is mentioned by Metchnikoff (238) but does not seem to have been found by any other investigator (Lotti (227), Achalme (5)). Phenol was found by Tissier (365), Tissier and Martally (366), and Loris-Mélikov (226).

Proteoses were mentioned by Tissier (365) and McCampbell (407) (from gelatin); proto- and deuterio-albumoses by Martin (233); peptones by McCampbell (407) (from gelatin); leucin by Tissier (365), and Schattenfroh and Grassberger (416) (in liquefied blood serum); and tyrosin by Tissier (365), Jungano and Distaso (169), and Martin (233).

Tissier (365) alone reports the presence of amines and urea.

Hydrogen sulphide was found by Tissier (365); Kolle and Hetsch (193) (in dextrose broth, serum, and milk); Jungano and Distaso (169); Schattenfroh and Grassberger (416) (occasionally from peptone, never from any other media); Fraenkel (91); Muscatello (246); and Korentchevsky (194, 195).

Ammonia was mentioned by Tissier (365), Jungano and Distaso (169) and by Herter (132); methyl mercaptan by McCampbell (407); putrescin, cadaverin, and methylenediamine by Mostînski (245); "aromatic oxy-acids" by McCampbell (407); and foul gases by Jungano and Distaso (169), and Schattenfroh and Grassberger (416) (from liquefied Loeffler's serum).

"Alcohol-soluble substances" were found by Martin (233).

Gas from the decomposition of proteid was noted by Hitschmann and Lindenthal (149) (in sugar-free agar only), Herter (132), Welch (389), and Ernst (77) (gas of a "sweetish odor").

Butyric acid is mentioned by Herter (132) (in "nearly sugar-free media"); and caproic and propionic acids by Schattenfroh and Grassberger (416).

No extensive chemical analyses were carried out with my cultures.

TOXINS.

On the question of toxin production by *B. welchii* there is the same confusion of statement that has been noted in all other phases of the study of this organism. Evidence from two sources, clinical and experimental, is adduced to support the contention that members of the group of *B. welchii* produce toxin. Hitschmann and Lindenthal (151), Schattenfroh and Grassberger (319), and Muscatello and Gangitano (248) stated that the general symptoms accompanying an infection with this organism are those of a severe toxemia. Fraenkel (89, 91) also thought that the death of the patient in such infections was due to toxic substances produced in and absorbed from the infected tissues.

Metchnikoff (237) declared that the toxin-producing powers of *B. welchii* are greater than those of *B. sporogenes* or *B. putrificus*. He found that toxin was produced in a mixture of chopped meat and water, the production being greatest from the second to the fifth days, the amount thereafter diminishing. Six to nine cubic centimeters of the filtrate from cultures of the gelatin-liquefying strain of *B. welchii* isolated by d'Agata (8) had a "fatal toxic action" upon guinea pigs. Passini (261, 262) produced a powerful toxin by growing cultures in a special medium composed of lean meat partially digested with trypsin. Korentchevsky (194) and Loris-Mélikov (225) also noted toxin production in their cultures. Brown (40) quotes Theobald Smith to the effect that *B. welchii* produces toxin in sugar-free broth containing a bit of fresh sterile tissue. Kamen (170), Hitschmann and Lindenthal (149), Jungano and Distaso (169), and Martin (233, 234) failed to find toxins in cultures of the strains examined by them.

This variation in results is due in part to a variation in the toxin-producing powers possessed by different strains. Metchnikoff (237) isolated an organism which did not produce toxin from a case of appendicitis; another strain derived from normal feces produced powerful toxin. Korentchevsky (194) observed the same difference in the results obtained with different strains. Schultze (332) noted that in some cases development of gas in the tissues after intravenous injections of *B. welchii* was accompanied by disappearance of the nuclei; in other cases the nuclei were present. He thought that in the first instance the organism used was very virulent and the disappearance of the nuclei due to a definitely toxic action of the bacteria, while the presence of the nuclei in the second instance was due to an absence of toxin formation. Heightening the virulence of a culture by passage does not appear to enhance its toxin-producing powers (169).

The character of the medium used is of importance in the production of toxin. Metchnikoff (237) and Korentchevsky (194), as already stated, used finely chopped lean meat in water, the mixture being sterilized in the autoclave. McCampbell (407) used dextrose broth. Passini (261) used a trypsin digest of beef muscle to which sugar had been added. He also found toxin in cultures in beef and horse serum, human ascites fluid, egg-white, brain substance, and "bronchial secretion," to which sugar (dextrose, lactose, saccharose, or maltose) had been added. Parallel tests with mother's and cow's milk showed toxin formation in the latter, but none in the former. No toxin was found in sugar-free media in which *B. welchii* always developed the sporulating form. If sugar was added to a five day old, sugar-free alkaline, non-toxic, putrid growth, the sporulating form immediately changed into a non-sporulating one, and toxic substance was rapidly developed. Such a culture remained alkaline (264). These results were not confirmed by McCampbell (407). If Passini's results are substantiated, the relation of sugar to the production of toxin by *B. welchii* differs radically from its relation to the production of toxins by *B. tetani* (414).

The stage at which toxin is reported to be most abundant in cultures varies greatly. Thus Korentchevsky (194) used cultures one to three days old; Metchnikoff (237) found toxins most abundant from the third to fifth days, the amount present diminishing after that time; toxins were produced in Passini's (261) cultures only after fourteen to thirty days. McCampbell (407) used filtrates from twenty-four hour dextrose broth cultures.

The character of the poison produced by *B. welchii* is not that of a true or soluble bacterial toxin. It is not destroyed by heating to 80° C. for more than an hour (McCampbell (407)); or to 100° C. for fifteen minutes (Passini (261), and Metchnikoff (237)). It deteriorates very slowly on standing (Metchnikoff (237)). It is dialyzable (Passini (261)). The symptoms of poisoning show themselves immediately after injection without the occurrence of any incubation period (Metchnikoff (237), Passini (261), Korentchevsky (194)).

The reaction of the toxin-containing filtrates has been almost certainly acid. Metchnikoff (237) and Korentchevsky (194) do not mention the reaction of the filtrates used by them, but since their cultures were grown in a suspension of chopped meat, it is reasonable to suppose that the reaction was acid. Passini (261), however, claimed that his 30 day old cultures in dextrose-trypsin-digest were distinctly alkaline, although they were at first acid.

McC Campbell (407) was convinced that the toxic effect of the filtrates of cultures of *B. welchii* was due entirely to the presence of butyric acid. He compared the results of the intravenous injections of filtrates of dextrose-broth cultures of *B. welchii* with the effect of intravenous injections of solutions of butyric acid of the same strength as the filtrate. The symptoms produced were identical in each case. The subcutaneous injections of butyric acid of similar strength frequently led to the production of phlegmons. The intravenous and subcutaneous injections of the neutralized filtrate were without effect. The toxic effects of butyric acid have been studied by Mayer and by Sternberg (345), who found that intravenous injections of small doses produced drowsiness, and larger doses caused coma and death; and by Karczag (171), who observed a definite toxic action of solutions of butyric acid upon a muscle-nerve preparation of a frog. But from the clinical symptoms which follow intravenous injections of filtrates of cultures of *B. welchii* it seems evident that the whole effect is not produced by butyric acid alone.

Passini (261) was unable to isolate any distinct poison from his cultures, but was convinced that his filtrates contained two separate toxic substances: one which caused sudden death in animals by injury to the respiratory center and circulatory organs; the other vomiting, diarrhea, and death in ten to twelve hours. There were two distinct types of intoxication. In one there were marked motor disturbances; the animal would run rapidly about the room, colliding with objects, and would later develop convulsions and die. In the other type the animal became paralyzed in the head and limbs in ten to twenty seconds and died shortly after. In dogs the toxin stimulated the smooth muscle of the intestine. Passini (261) quoted unpublished work of Lindenthal which showed that the toxin of *B. welchii* acts upon the musculature of the uterus causing violent contractions.

The symptoms described by Metchnikoff (237) and Korentchevsky (194) as following injections with toxins of *B. welchii* differed in some respects from those given by Passini. Korentchevsky found young animals much more susceptible to the action of the poison than older ones. The symptoms caused by intravenous injections into rabbits developed in from one to three hours. There was first dyspnea, then paresis of the muscles of the extremities, and sometimes with convulsive movements of the head. In two and one-half to four and one-half hours violent convulsions occurred followed usually by complete paralysis. Korentchevsky obtained toxic effects from his filtrates whether injected intravenously, fed by mouth, or injected into the rectum. He found that a strain of

B. welchii which produced sufficient toxin to cause the death of a rabbit by intravenous injection would also kill a young animal of the same species if injected into the rectum. The rabbits injected per rectum lost weight, became very weak, and usually died if the injections were continued.

Loris-Mélikov (225), who studied the relation of anaerobic bacteria to the ulceration of Peyer's patches in typhoid fever, states that *B. welchii* produces a toxic substance which acts especially upon the lymphoid tissues of the body. Intraperitoneal injections caused the death of the animal. At autopsy Peyer's patches were hyperemic and swollen, but never ulcerated.

Tissier (362) and Savchenko (314) think that the toxins of *B. welchii* are capable of causing necrosis.

None of my cultures which were tested for toxin production showed evidence of producing any poisonous substances in artificial media. Organism 1 of this series, isolated from a normal stool, was studied more carefully than any of the others. This organism was quite pathogenic for guinea pigs. The following experiments were carried out with this organism.

(a) Whey from a four day old milk culture was passed through a Chamberland filter. This filtrate was titrated and found to require 2.6 c.c. of $\frac{N}{1}$ sodium hydrate to neutralize 100 c.c. It was then divided into two parts and one was exactly neutralized with $\frac{N}{1}$ sodium hydrate. 6 c.c. of the neutralized filtrate were injected intraperitoneally into a guinea pig weighing 290 gm. There were no signs of discomfort and no symptoms of any kind following the injection. 10 c.c. of the unneutralized filtrate were similarly injected into a guinea pig weighing 530 gm. Restlessness developed which lasted about twenty minutes. There were no further symptoms.

(b) Two cultures—one pure, the other in symbiosis with *B. prodigiosus*—were grown for four days in sugar-free broth containing bits of sterile guinea pig spleen. They were then passed through a Chamberland filter. 5 c.c. of the filtrate from the pure culture were injected into the peritoneal cavity of a guinea pig weighing 470 gm. and 3 c.c. of the filtrate from the mixed culture into the peritoneal cavity of a guinea pig weighing 330 gm. In neither instance was there the slightest indication of poisoning.

Cultures of twelve strains were examined for toxins after growing in sugar-free broth containing coagulated egg-white. Growth in this medium, according to Tissier (365), heightens the virulence of the culture. The broth was centrifugalized and the clear supernatant fluid injected subcutaneously. In some instances the fluid still contained living virulent bacilli and a local infection followed. The results are shown in Table VI.

Endotoxins.

McC Campbell (407) injected emulsions of *B. welchii*, which had been ground in a mortar, in doses of 0.1 to 1 cubic centimeter intravenously

TABLE VI.

Results of Examination for Toxin of Cultures of Bacillus welchii in Sugar-Free Broth Plus Coagulated Egg-White. (Broth Centrifugalized, not Filtered.)

Organism No.	Source.	Age of culture.	Amount injected.	Weight of guinea pig.	Immediate effect.	Final result.
		dys.	cc.	gm.		
1	Stool, normal	6	6	350	None	None.
2	Soil	25	6	380	None	None.
4	Stool, typhoid	7	6	340	None	None.
25	Cow feces	26	6	350	None	None.
3	Soil	7	6	375	Slight discomfort, from which animal soon recovered	None.
5	Potato	13	5	325	Slight discomfort, from which animal soon recovered	None.
6	Appendix, normal	6	6	400	Restlessness	None. Slight induration at site of injection. Disappeared in 3 dys.
19	Stool (infant), diarrhea	6	6	320	Restlessness	Induration 3 x 4 cm. at site of injection. No ulceration. Disappeared in 7 dys.
21	Stool (infant), normal	24	6	400	Restlessness	Swelling 3 x 5 cm. at site of injection. Hair over swelling slips rather easily. No ulceration. Disappeared in 8 dys.
23	Stool (adult), pernicious anemia	26	5	320	Slight discomfort	Induration 2 x 3 cm. Disappeared in 4 dys.
20	Stool (infant), normal	6	6	380	Some discomfort	In 24 hrs. crepitating swelling on entire under surface of abdomen. Found dead 36 hrs. after injection.
22	Stool (adult), diarrhea	24	5	400	Some discomfort	Large crepitating swelling on abdomen. Died 21 hrs. after injection.

and intraperitoneally. No effects were produced in rabbits, and only very slight indications of intoxication were evident in guinea pigs. The intraperitoneal injection into rabbits of very large doses of eight (Nos. 1, 2, 6, 19, 20, 21, 22, and 23) of my strains killed either by heat or by several hours' exposure to air, caused no symptoms of any kind.

Leucocidins.

Hitschmann and Lindenthal (149), Savchenko (314), and others have noted the comparatively small number of polymorphonuclear leucocytes in the serohemorrhagic exudate present in local infections with *B. welchii*. Among several cases of infection with *B. phlegmonis emphysematosæ*, it was only in a case of meningitis that Hitschmann and Lindenthal found true pus. Savchenko found that the bacillus of Achalme (*B. welchii*) secreted a substance which caused a negative chemotaxis. McCampbell (407) observed that no phagocytosis occurred *in vitro* unless the bacilli were washed free of acids which neutralize the opsonins and cause changes in the leucocytes. Kamen (170) describes leucocidins in dextrose broth cultures (methylene blue test).

Hemolysins.

Evidence of the hemolytic powers of *B. welchii* has been noted clinically. Klotz and Holman (192), Leroy (429), and others have called attention to the rapidly developing anemia which usually accompanies infections with *B. welchii*. One of Schultze's (332) infected rabbits developed a hemoglobinuria which he believed to be due to the action of a hemolysin acting *intra vitam*. Herter (132) observed that "rabbits injected with pure cultures (of *B. welchii*) killed and incubated at 37° C. soon show indications of hemolysis." He also noted advanced hemolysis in the bodies of persons dead of gas bacillus infections. Schumm (473) found a band corresponding to hemoglobin on spectroscopic examination of the serum of a patient with *B. welchii* septicemia.

Ability to produce hemolysins varies greatly among different strains of *B. welchii*. In Korentchevsky's (194) cultures they were either absent or so small in amount as not to interfere with a complement fixation test.

Herter (132) and Hewlett (141) found hemolytic substances in blood bouillon; Kamen (170) observed them in dextrose broth cultures. McCampbell (407) believed that the hemolysis caused by cultures in dextrose broth was due to the presence of butyric acid. He showed that the same degree of hemolysis can be produced by solutions of butyric acid of the same

titer as the broth culture. The clear solution resulting from the hemolysis in his experiments was reddish brown in color instead of the bright red seen in true hemolysis. McCampbell also found that for a short time after being injected with cultures of *B. welchii* the serum of rabbits caused an "atypical hemolysis," caused, he thought, "by the salts of the acids produced in the cultures, the salts being very slowly eliminated."

TABLE VII.

The Hemolyzing Power of Seventeen Strains of Bacillus welchii. Cultures Grown Anaerobically in Sugar-Free Broth Containing Coagulated Egg-White.

Organism No.	Age of culture.	1 c.c. erythrocyte suspension plus					Source of organism.
		1 c.c. culture. 0 c.c. sodium chloride.	0.5 c.c. culture. 0.5 c.c. sodium chloride.	0.3 c.c. culture. 0.7 c.c. sodium chloride.	0.2 c.c. culture. 0.8 c.c. sodium chloride.	0.1 c.c. culture. 0.9 c.c. sodium chloride.	
	dys.						
2	8	+++	+++	++	++	+	Soil (Brenham, Texas).
3	5	+++	+++	++	++	+	Soil (Boston, Mass.).
6	5	+	—	—	—	—	Normal appendix, autopsy.
17	40	+++	+++	++	+	+	Boston sewage.
19	8	+++	+++	+++	++	++	Diarrheal stool (infant).
20	6	+++	+++	++	++	+	Normal stool (6 day old infant).
21	5	+++	++	+	—	—	Normal stool (10 day old infant).
22	8	+++	++	++	+	+	Diarrheal stool (adult).
23	7	+	+	—	—	—	Diarrheal stool, pernicious anemia.
24	10	—	—	—	—	—	Diarrheal stool (infant).
25	9	—	—	—	—	—	Cow feces.
26	40	—	—	—	—	—	Cow feces.
27	40	++	+	—	—	—	Cow feces.
28	7	—	—	—	—	—	Stool, pernicious anemia (adult).
29	7	+	+	—	—	—	Stool, pernicious anemia (adult).
30	7	+++	++	+	+	—	Stool, pernicious anemia (adult).
31	7	+++	+++	++	+	—	Milk.
Control		—	—	—	—	—	Broth used instead of "culture."

+++ = complete hemolysis.
+ = trace of hemolysis.

++ = partial hemolysis.
— = no hemolysis.

Herter (132) states that "treatment of the filtrate in an exhaustion apparatus very slightly reduced the hemolytic action; heating to 70° C. for one hour reduced it still further; but even boiling did not wholly destroy it."

My own experiments show that some strains of *B. welchii* produce hemolysins in neutral or very slightly acid or alkaline broth, while others do not.

The source from which a strain was isolated is no indication of what its hemolyzing power will be. I found hemolysins produced in sugar-free or plain broth. Experiments with filtered whey from milk cultures of some of my strains showed that the acid whey was hemolytic but the neutralized whey possessed no hemolyzing power whatever. The results of my experiments are shown in Table VII.

SYMBIOSIS AND ANTIBIOSIS.

The symbiotic and antibiotic relations of *B. welchii* have not been extensively studied. Passini (264) believed that *B. welchii* produced spores quite readily when grown in symbiosis with *B. coli*. Sittler (340) thought that some cases of enterocatarrh are due to symbiotic relations between these organisms. Baup and Stanculeanu (22) were convinced that the presence of *B. coli* in the same lesion with *B. welchii* resulted in increasing the virulence of both organisms.

Seliber (334) thought that greater amounts of butyric acid were produced in cultures of *B. butyricus* and *B. perfringens* (*B. welchii*) than in cultures of *B. butyricus* alone or in symbiosis with *B. putrificus*.

Rosenthal (298) studied the symbiotic relations of *B. welchii* and *B. bulgaricus* in milk without coming to any definite conclusions.

Loris-Mélikov (225) injected mixtures of *B. perfringens* (*B. welchii*), *B. satellitis*, and *B. typhosus* into the peritoneal cavity of rabbits (?) and found that this caused the rapid death of the animal. At autopsy, in addition to the peritonitis, he reports the swelling and hyperemia and sometimes ulceration of Peyer's patches.

Distaso (66) grew *B. welchii* in symbiosis with *B. bifidus* and *B. acetogenes*. He found that there was some growth of *B. welchii* in cultures with *B. bifidus*, but no detectable growth in those with *B. acetogenes*. From the former *B. welchii* disappeared in less than ten days, from the latter in less than five days. Tissier (364) states that under certain conditions *B. bifidus* inhibits the growth of *B. welchii*.

Sittler (340) observed a marked antagonism between enterococcus and *B. welchii*.

Rosenthal and Chazarain-Wetzel (303) claim to have demonstrated an antagonism between the "*variété banale*," and the "*variété rhumatismale*" of *B. perfringens*.

Loris-Mélikov and Ostrovsky (226) stated that there is an antagonism between *B. welchii* and *B. tuberculosis*, so that if a guinea pig is injected with a mixture of these two organisms, no infection follows; if injected

with tubercle bacilli and later with *B. welchii*, infection with tuberculosis follows. *In vitro* *B. welchii* was thought to exert a bactericidal action upon *B. tuberculosis*.

RESISTANCE OF BACILLUS WELCHII.

Cultures in carbohydrate media die out very quickly. Even when protected from the air the accumulation of acid causes the death of the culture. Tissier (361) found that from 2.45 to 3.43 per cent. acid was necessary to arrest the growth of *B. welchii* in cultures growing in sugar media. Lotti (227) found that the accumulation of metabolic products caused the death of his cultures in two to three days. He was able to keep them alive in a dialyzer for twenty days. Achalme (3) claimed that 0.1 per cent. of salicylic acid was sufficient to prevent all growth of the bacillus isolated by him from a case of rheumatism.

Exposure to the air also results in quick killing of the culture. This occurs more promptly if the culture is kept in the incubator than if grown at room temperature. Gelatin cultures exposed to air do not die as quickly as agar cultures (Fraenkel (91)). Welch (389) and Pratt and Fulton (271) have called attention to the prompt loss of vitality by *B. welchii* in bodies kept in cold storage.

Cultures which contain spores can be preserved for many weeks or months. Jacqu  (165) preserved spores for five months. Flexner (85) found one of the cultures from the liver of H. U. Williams's case viable after eight weeks, and Loris-M likov (223) obtained growth from spores which had been kept protected from the air in egg-broth for five years.

The reported powers of resistance to heat show great variation. Thus spores in Dunham's cultures resisted 94  C. for one minute but were killed in five minutes. Andrewes (10) found spores of *B. enteritidis sporogenes* in rice pudding which had been cooked at a temperature ranging from 70  C. to 98  C. The time was not stated. McCampbell (407) found that the thermal death point for spores in his cultures was 100  C. for fifteen minutes. Rodella (288) and von Hibler (146) report strains, spores of which resisted boiling for an hour and a half. Wild (396) found the spores in stools much more resistant to heat than those in cultures.

VIRULENCE.

The virulence of different strains of *B. welchii* for laboratory animals varies greatly. All strains which have shown any virulence whatever are most pathogenic for guinea pigs. Rabbits are much more resistant to infection with *B. welchii* than are guinea pigs. Hom n (153), however, was

able to increase greatly the virulence for rabbits by passage through a series of these animals. Mice and rats (Hitschmann and Lindenthal (149), Werner (392), and Fraenkel (92)) are frequently refractory to *B. welchii*. Pigeons (Gwyn (118), Howard (160), Welch (389)) and sparrows (Fraenkel (92), Welch (389)) are quite susceptible; doves (Fraenkel (92)) only slightly so. *B. welchii* also produces a "typical inflammatory process" in dogs (Kolle and Hetsch (193)). Klein (184) attempted to classify the degrees of pathogenic power as follows: A strain of normal virulence was one, the subcutaneous injection of one cubic centimeter of the whey of a twenty-four hour milk culture of which, killed a guinea pig weighing 200 to 300 grams in twenty-four to thirty hours. One cubic centimeter of whey from a twenty-four hour milk culture of an organism of decreased virulence caused only extensive swelling with gas formation around the site of the injection or killed the animal only after three to four days. Less virulent strains may cause only swelling without gas formation, a condition observed clinically in man by Bloodgood (32), Clark (51), Spitta (343), Hosemann (155), and Williams (397).

The source from which an organism was isolated is not evidence as to its pathogenic properties. In almost every instance, strains of *B. welchii* isolated from lesions in man have been highly pathogenic for guinea pigs. Hosemann (155), however, isolated from an infection without gas formation in man a strain of *B. welchii* which was only capable of producing a local swelling without gas production when injected subcutaneously into guinea pigs. Werner (392) found that a strain of *B. welchii* from a fatal case of infection was less virulent for guinea pigs than a strain from a non-fatal case.

Klein (182) found virulent strains of *B. enteritidis sporogenes* in sewage. Klein (182) and Passini (262) isolated both virulent and avirulent strains from normal and diarrheal stools. Schattenfroh and Grassberger (318) isolated eight non-virulent strains of *B. welchii* from market milk, and one very virulent organism from the soil.

Of the eleven organisms of my series which were tested for virulence, seven were found to be virulent. They were isolated from the following sources:

Normal stool (infant).....	2
Normal stool (adult).....	1
Diarrheal stool (infant).....	1
Diarrheal stool (adult).....	1
Stool (adult), pernicious anemia	1
Normal appendix, autopsy.....	1

The four strains which were not virulent were isolated from:

The soil.....	2
Stool (adult), typhoid fever.....	1
Cow feces.....	1

Various conditions to which the organism is subjected influence its virulence. Kamen (170) believed that the heating of the spores in the method ordinarily used in isolating *B. welchii* may render some strains avirulent. Prolonged cultivation of *B. welchii* on artificial media causes reduction of virulence. The medium on which the organism is grown appears to influence its pathogenic powers, but in just what way is not clear. Thus Hitschmann and Lindenthal (149) state that virulence of *B. phlegmonis emphysematosæ* varies with the acid and gas formation, and is, therefore, greatest in media containing sugar. Tissier (362) and Rosenthal (301), on the contrary, found that their strains of *B. perfringens* (*B. welchii*) showed greater virulence if grown in sugar-free broth containing coagulated egg-white. Brown (40) quotes Theobald Smith to the effect that cultures of *B. welchii* in sugar-free broth plus sterile tissue would cause infection in guinea pigs, while cultures of the same strain in broth without tissue did not cause infection. My own cultures showed greater virulence in sugar-free broth with coagulated egg-white than in any other medium. Organism 22 of my series was very highly virulent. Five cubic centimeters of the clear supernatant fluid of a twenty-four day old culture in broth plus egg-white after thirty minutes' centrifugalization still contained enough bacilli to kill a 400 gram guinea pig in twenty-one hours.

Passini (261, 262) believed that the sporulating and non-sporulating forms of *B. welchii* differ in virulence and in the type of lesion produced. The former produced little or no gas in the tissues, and the exudate was more hemorrhagic in character than in the case of the non-sporulating form. The very virulent culture (No. 22 of my series) mentioned above contained spores. The skin of the guinea pig was loosened from the subjacent muscles from axillæ to inguinal region by gas formation.

The presence of toxic products or of other organisms in symbiosis may also condition the virulence of *B. welchii* (de Gasperi and Savini (99) and Gehrmann (100)). One of my strains, isolated from the stool of a young girl with typhoid-like symptoms killed a guinea pig in five hours after intraperitoneal injection. On opening the peritoneal cavity within ten minutes after the death of the animal, *B. coli* and *B. welchii* were found in the peritoneal fluid. There was no gas. The exudate was very poor in

leucocytes and was hemorrhagic in character. The parietal and visceral peritoneum was intensely hyperemic.

Certain conditions determine whether infection will take place in a guinea pig injected with *B. welchii*. The size of the dose is important, especially with organisms of moderate or low virulence. Rosenthal (301) was able to cause infection with large doses only. Klein (184) inoculated four tubes of milk with 1/100, 1/50, 1/10, and 1/5 of a cubic centimeter, respectively, of sewage, heated to 80° C. for fifteen minutes and incubated. All gave a typical reaction for *B. welchii*. One cubic centimeter of the whey from each tube was injected into guinea pigs. Only the whey from the tubes inoculated with 1/10 and 1/5 of a cubic centimeter of sewage showed normal virulence; *i.e.*, killed the animal in twenty-four to thirty hours.

Fraenkel (92) was of the opinion that *B. phlegmonis emphysematosæ* could cause infection if only it gets under the skin. Westenhoffer (394) and Muscatello and Gangitano (247) and others are convinced that *B. welchii* can grow only in dead tissues or tissues which have had their vitality altered.

Mixed cultures are more quickly and uniformly fatal than pure cultures of *B. welchii* alone. In Little's (220) experience puerperal pure infections with *B. welchii* were mild. The fatal cases were mixed infections. Heinrichus (128) thought that pregnancy appeared to favor pathogenic action of *B. welchii*, "because the degeneration of epithelial cells in the uterus which accompanies pregnancy enables this organism to find entrance in the tissues."

Young animals are much more susceptible than older ones. Intra-peritoneal injection is much more uniformly and promptly fatal than subcutaneous.

Hitschmann and Lindenthal (151) believe that *B. welchii* is very quickly killed off in the circulating blood and that infection spreads along the lymphatics. But *B. welchii* has been cultivated from the blood during life by Lenhartz (211), Gwyn (118), Rocchi (421), Hewitt (137), Roger and Garnier (289), and Baugher (474). Lindenthal (217) was of the opinion that *B. welchii* can grow in the blood only when the heart action becomes so weak that the blood is largely venous throughout the body.

The lesion produced by artificial infection of a guinea pig is quite characteristic. There is formed a more or less extensive bladder-like elevation of the skin from the underlying tissues with an accumulation of gas and fluid which contains many bacilli and is sometimes rich in cells. There is a pronounced degeneration of the neighboring muscle tissue. The hair over such an affected region slips very easily.

INFECTIONS DUE TO *BACILLUS WELCHII*.*Eye, Ear, and Nose.*

Schumacher (333), Darier (60), and Lutz (229) have each reported a case of panophthalmitis due to *B. welchii*. The bacteriological examination in the first two instances was not above criticism. Roemer (292) and Hanke and Tertsch (440), and Stanculeanu and Baup (344) found this organism associated with infections of the eye. Benedetti (26) and Chailus (47) each reported a case of infection of the vitreous humor with *B. welchii*.

Stanculeanu and Baup (344) claimed that those infections of the antrum of Highmore which come from carious teeth are always associated with fetid pus and anaerobic bacteria, among which *B. welchii* is frequently found. This harmonizes with Baumgartner's (21) frequent finding of *B. welchii* in carious teeth. Turner and Lewis (214) found anaerobic bacteria in 19 out of 43 cases of acute infection of the accessory sinuses of the nose, and in 11 out of 35 cases in another series (371). Among these *B. perfringens* (*B. welchii*) was "a most important representative."

Rist (280) isolated *B. perfringens* (*B. welchii*) from cases of otitis media. Baup and Stanculeanu (22) found it associated with *B. coli* in infection of the middle ear with thrombosis of the lateral sinus. Hudson (161) found *B. welchii* in pus from the ear in a case of mastoiditis, otitis media, empyema of the antrum of Highmore and a "necrotic tooth."

Meninges and Brain.

Hitschmann and Lindenthal (149) described a case of meningitis due to *B. phlegmonis emphysematosæ* following fracture of the base of the skull. Howard (160) reported a case of meningitis with gas cysts in the brain caused by *B. aerogenes capsulatus* following an operation on a perineal fistula. In both cases typical pus was formed.

Reuling and Herring (278) found *B. welchii* in pure culture in cavities of the brain at autopsy after death from a gunshot wound of the abdomen. Falkner (79) reports two cases of gas cysts of the brain associated with *B. welchii*. Siemerling (336) and Madison (425) each discovered gas cysts due to *B. welchii* in the brain at autopsy on patients with general paresis. It is likely that the formation of the cysts in the last five cases was largely a postmortem occurrence.

Lungs and Pleura.

Levy (212) described a case of pneumothorax, and Nicholls (252) and Hamilton (427) a case of pyohemopneumothorax associated with *B. welchii*.

Rendu and Rist (452) reported a case of putrid pleurisy due to *B. perfringens* (*B. welchii*). Rist and Ribadeau-Dumas (282) produced a putrid pleurisy in guinea pigs by the injection of cultures of *B. welchii* into the pleural cavity.

Reinbach (275) found *B. welchii* in pure culture in a case of gangrene of the lung. Babes (441) found it in a similar case with staphylococci, streptococci, and *B. coli*. Rist (281) isolated *B. perfringens* (*B. welchii*) from "gangrenous pneumonia." In 10 cases of gangrene of the lung reported by Guillemot (115) in which the bacteriological examination was made, *B. welchii* was found in one. Welch and Flexner (390) reported two cases in which *B. welchii* was found in an infarction of the lung.

Wright and Stokes (475) found *B. welchii* in pus from abscesses of the liver and in fibrinopurulent pericardial exudate in a steer found to have a long needle penetrating the heart from the stomach.

Gastro-Intestinal Tract and Liver.

Schultze (332) has reported a death due to gastric hemorrhage from multiple ulcerations of the stomach. In these ulcers *B. welchii* was present in great numbers. Schultze believed that *B. welchii* was causally related to the formation of the ulcers. This conclusion was vigorously disputed by Braun in the discussion of Schultze's paper. Howard (160) noted "superficial ulcerations of the intestinal mucosa" at autopsies upon four bodies showing "*Schaumorgane*." Loris-Mélikov (225) believed that *B. welchii* played a very important part in the swelling, but not in the ulceration of Peyer's patches in typhoid fever.

Rist (281) found *B. welchii* in a gall bladder almost obliterated by a stone. Gilbert and Lippmann (104) isolated this bacillus from the gall bladder in 16 per cent. of the cases of cholecystitis examined by them. Rist and Ribadeau-Dumas (282) were able to produce abscess of the liver and angiocholitis by intravenous injection of *B. welchii*. Stolz (349) reports two cases of gas bacillus infection of the bile passages. The production of the gas in the liver he thought occurred post mortem. Welch (388) noted *B. welchii* in gas blebs on the liver of a pig.

B. welchii has been isolated from inflamed appendices, but its etiological relation to appendicitis is by no means settled. Its presence in infections of that organ was first noted by Veillon and Zuber (380). They gave it the name of *B. perfringens*. Krogus (197) found bacilli which he considered *B. welchii* "frequently in smears from the pus" of appendiceal abscesses. Grigoroff (403) studied the bacterial flora of 18 normal and of 31 diseased appendices. In the normal specimens aerobic and anaerobic

bacteria were present, either in approximately equal proportion or aerobic organisms were the more abundant. In the inflamed appendices, however, the strictly anaerobic bacteria were strikingly predominant. In the 31 cases of appendicitis, *B. perfringens* (*B. welchii*) was found 9 times. Lanz and Tavel (201) in 138 cases of appendicitis found *B. edematis maligni* in 49. These authors classed under the name *B. edematis maligni* a variety of anaerobic bacteria including *B. welchii*, *B. chauvei*, and the true *B. edematis maligni*. In 26 cases of appendicitis Heyde (142) found that anaerobes were more numerous than aerobes in 25. In 10 cases the anaerobes were purely fermentative organisms (motile and non-motile butyric acid bacilli, *B. edematis maligni*, and the putrefactive butyric acid bacteria). Of these 10 cases 4 were children, of whom three died. Only 4 of the 10 cases were fatal. The symptoms and pathologic findings were "like those of a progressive gas phlegmon." In another series (435) of 102 cases, including 30 of purulent and 21 of gangrenous appendicitis, 27 of peritonitis, 9 of "abscess," and 15 of "chronic epityphlitis and internal operations," Heyde found various anaerobes in all except 6 cases, which gave pure aerobic cultures. *B. perfringens* (*B. welchii*) was isolated 9 times from the 48 cases of gangrenous appendicitis and peritonitis. Perrone (267) found *B. welchii* in 6 out of 14 cases of appendicitis, always associated with other bacteria. Runeberg (306) obtained *B. welchii* from only one out of 14 cases of appendicitis. He believed that anaerobes played an important part in peritonitis and appendicitis by the production of toxins rather than by actually taking part in the infection. Metchnikoff (237) attempted to produce appendicitis in chimpanzees and only succeeded in one instance in which *B. welchii* was used.

The relation of anaerobes in general and of *B. welchii* in particular to appendicitis is still unsettled. Heyde (435) believed that they play a very important part. He found in a series of cases of appendicitis that anaerobes, among which was *B. welchii*, were especially abundant at the beginning and at the advancing margin of the infection. He found that anaerobes were able to penetrate the wall of the appendix more quickly than aerobes, with which finding the results of the work of Ikonnikoff (162) agree. This phenomenon may account for the presence of the sterile pus sometimes found in the peritoneal cavity before rupture of the appendix itself.

In a specimen of pus from an appendix from the clinic of Dr. J. É. Thompson, examined by me, *B. welchii* was present in the sporulating form. The same appears to have been the case in other instances reported in the literature. I also found spores of *B. welchii* in 90 per cent. of normal appendices obtained at autopsy. They were present in approximately

100 per cent., if the appendix contained fecal matter. Hitschmann and Lindenthal (149) have called attention to the fact that *B. phlegmonis emphysematosæ* (*B. welchii*) never forms spores in tissues in which it is playing a pathogenic part. It is possible, therefore, if spores of *B. welchii* are present in appendiceal pus, that its presence is only accidental and not in any way connected etiologically with the infection.

Dudgeon and Sargent (70) did not believe that anaerobic bacteria played any important part in appendicitis or peritonitis. They believed that if an organism like *B. aerogenes capsulatus* was frequently present in cases of appendicitis, emphysematous gangrene of the bowel should be a common lesion instead of an interesting curiosity, and that foaming viscera should be seen very frequently at autopsies on patients dead with appendicitis. Instead, there was only one case of foaming organs in such cases among the autopsies at St. Thomas's Hospital in one year (1904).

Flexner (86) found *B. welchii* in 8 out of 60 cases of endogenous peritonitis, usually associated with other organisms. Hitschmann and Lindenthal (149), von Hibler (147), and Wright and Stokes (475) isolated this organism in pure culture from peritonitis complicating typhoid fever. Welch (389) collected 13 cases of peritonitis in which *B. welchii* was associated with other organisms. Pratt and Fulton (271) found it in a case of peritonitis originating from a perforated gastric ulcer. Manahan (231) reported *B. welchii* in smears of the pus from 4 out of one series of 19 cases of appendicitis and cultivated it from only one of a second series of 89 cases (230). Ghon and Sachs (102) stated that they had found *B. welchii* in cases of peritonitis "on several occasions." Welch and Flexner (390) have reported 8 cases of peritonitis in which *B. welchii* was found in the peritoneal exudate as follows: in pure culture in a case of perforation of an ulcer of the pylorus; mixed with other organisms in 3 cases of perforation of typhoid ulcers; in one case each of strangulation of the intestine; infarction of the intestine; in the peritoneal fluid without perforation of the intestine, probably postmortem invasion; spontaneous ulcer of the stomach with perforation in a rabbit. Von Hibler (147) reported 3 cases of peritonitis associated with *B. welchii* after operation for cancer of the kidney, necrosis of a sarcoma nodule in a lymph gland, and the breaking of a stomach fistula.

On the other hand, Dudgeon and Sargent (70) made careful examinations for anaerobic bacteria in 270 cases of peritonitis and obtained positive results in only one case from which they isolated *B. welchii*. Fishbein (83) found *B. welchii* in only one out of 145 cases of peritonitis.

The relation of *B. welchii* to diarrhea will be discussed later.

Genito-Urinary Organs.

Williams (397) found *B. welchii* in the pus from a suppurative pyelitis in which there was absence of gas formation.

It might reasonably be expected that infections of the genito-urinary organs by *B. welchii* would occur more frequently than they have been reported. The proximity of the external genitalia to the lower end of the gastro-intestinal tract favors infection. Puerperal sepsis due to gas bacillus has followed attacks of diarrhea coming on immediately before or after labor (Halban (120)). Gas phlegmon of the perineal tissues and scrotum has had its origin in hemorrhoids (Gilpatrick (447)). In addition to this source the vagina itself not infrequently contains gas bacilli (Hallé (121), Kronig and Menge (198), Schild (326), Sittler (340), and Scheidler (325)). Scheidler believes *B. welchii* to be a normal inhabitant of the vagina, a statement which is denied by Young and Rhea (446).

Lindenthal (216) found gas cysts in the wall of the vagina which were due to an organism probably identical with *B. welchii*, but described by him as *B. vaginæ emphysematosæ*. The same author reported a case of tympania uteri (217, 218) caused by a similar bacillus. Scheidler (325) reported a case of death following curettage in which there were no gross changes in the uterus except those due to the operation, but *B. welchii* was found in the uterus at autopsy.

B. welchii may be the cause of puerperal sepsis either after normal labor or after abortion. Kronig and Menge (198) found *B. welchii* in the amniotic fluid in two instances. Levy (213) reported a case of gas abscess from postpartum endometritis. Dobbin (67), Holmsen (152), and Ernst (77) each described a fatal case of puerperal sepsis due to *B. welchii* in which "Schaumorgane" developed post mortem. A case of puerperal sepsis with cystitis, pyelitis, and pyelonephritis reported by Cesaris-Demel (46) was probably caused by *B. welchii*. Of Little's (220) ten cases of febrile puerperiums, in two *B. welchii* was present in the lochia. In one of these there had been a version and extraction, in the other one antepartum vaginal examination. Hüssy (404) described a similar case with *B. welchii* and streptococcus in the lochia. Halban (119, 120) has reported two cases of fatal gas bacillus sepsis following instrumental delivery. In one case the patient suffered from a severe diarrhea just previous to the onset of labor (120). At autopsy in both instances there was abundant gas formation throughout the body. Jeannin (167) saw a case of puerperal sepsis following normal labor in which there was metastatic infection of a concomitant pleurisy and bronchopneumonia. Fromme (94) has reviewed the literature on puerperal sepsis and mentioned cases of his own due to *B.*

welchii. Wright and Stokes (475) saw a gas bacillus and streptococcus infection in a woman who had been for four days in labor, a podalic version having been performed.

Graham, Steward, and Baldwin (109) have described a remarkable case of death from gas bacillus sepsis following abortion in which the body of the patient became greatly swollen from gas before death. Lenhartz (211) reported a case of puerperal sepsis following abortion in which *B. welchii* was seen in blood smears taken two hours before death, and in which there was general gas formation throughout the body after death. Kierle (178), from a long experience as coroner's physician in Baltimore, found that the phenomenon of "*Schaumorgane*" was "not infrequent in cases of death from criminal abortion." Braun, in discussing Halban's (119) case, described one of his own in which death from gas bacillus infection followed an attempted criminal abortion. Whitacre (423) and Dreyfoos (424) each reported cases of probable infection with *B. welchii* after abortion, although no bacteriological examination was made. Young and Rhea (446) reported two fatal cases of gas bacillus sepsis after abortion. Both patients of Young and Rhea and the one of Dreyfoos showed a peculiar purplish changing to bronze discoloration of the skin.

Scheidler (325) found *B. welchii* in the pus from the cul-de-sac in an infection following abortion. In one series of 100 cases of abortion Schottmüller (327) found *B. welchii* in the blood and lochia of five. In one instance *B. welchii* was present in pure culture; the lochia was not foul and the patient recovered; in two others *B. welchii* was associated with other organisms (staphylococcus and pneumococcus, and *B. coli*, respectively), and the placenta gave off a foul odor. One patient recovered, the other died. In another series of 145 cases of abortion, Schottmüller (328) found *B. welchii* in the blood and lochia of five. Roemer (292) made blood cultures in 131 cases of abortion, and *B. welchii* was fifth in the order of frequency of the organisms found. Warnekros (383) obtained positive results from blood cultures in eleven out of twelve cases of abortion. In two of these eleven cases *B. welchii* was obtained from the blood. Only one of the eleven patients died, a mixed infection by *B. welchii* and streptococci. Warnekros is of the opinion that entrance of bacteria into the blood is much more likely after abortion than after normal labor, because of the difference in the manner of separation of the placenta in the two conditions.

Infections with *B. welchii* of the male genital organs have been reported following external urethrotomy (2 cases) and injury to the urethra (Dunham (71)); periurethral suppuration (Cottet (57)); passage of a catheter (Robertson (283)); periurethral gangrene and abscess and gangrene of the bladder (Jungano (405)); suppuration of the prostate (Cottet and Duval

(406)); and drainage of the bladder through the perineum (Stewart (422)). Welch and Flexner (390) reported two cases of pyonephritis and pyonephrosis resulting from infection induced by stricture of the urethra and catheterization, and hypertrophy of the prostate, respectively, in which *B. welchii* was found associated with pyogenic organisms. Welch and Flexner (390) report two cases, Flexner (quoted by Welch (389)) one case, and Kelly and McCallum (454) one case of gas in the urinary bladder due to *B. welchii*.

General Septicemia.

B. welchii appears to be able to cause a very rapidly fatal form of septicemia without any evident point of entry into the body. Welch and Flexner (390) reported the case of a negro epileptic who was found in an unconscious condition with frothy, bloody fluid exuding from the mouth and nose. He died within twenty minutes, and an hour after death his body was greatly swollen with gas produced by *B. welchii*. Hamilton and Yates (124) reported a case of purpura hemorrhagica of extreme severity, in which *B. welchii* was found in the liver and other organs of the body at autopsy. Bernhardt (27) described the case of an idiot who suddenly became violently ill. He had profuse diarrhea and scanty, bloody urine, and died thirty-six hours after the onset. Autopsy revealed bronchopneumonia, and gas in the heart and all organs of the body due to the gas bacillus. Boni (33) reported a rapidly fatal septicemia originating apparently in a peritonsillar abscess. Thaon (355) related the history of the case of a man who fell into a cesspool, and without any external injuries very soon thereafter showed symptoms of septicemia and died in twenty-three hours. At autopsy, six hours post mortem, *B. perfringens* (*B. welchii*) was isolated from the heart's blood in pure culture. Owen and Glynn (443) reported a remarkable case of a woman who, six hours after eating canned salmon, was attacked by severe pains in the lower part of the abdomen, with vomiting, diarrhea, and rapid development of signs of severe toxemia. There was puffiness under the eyes, the skin became mahogany brown in color, and the urine was "like porter." At autopsy, fifteen hours post mortem, the body was swollen beyond recognition, and *B. welchii* was present in all parts of the body.

Flexner (470) in a study of terminal infections found evidence that the gas bacillus may sometimes invade the body shortly before death.

Emphysematous Gangrene and Gas Phlegmon.

The literature dealing with these types of infection by *B. welchii* has recently been reviewed thoroughly by Stewart (422). He collected sixty-

one cases which showed a mortality of 55 per cent. The methods of treatment and the character of the descriptions, clinical and bacteriological, of such cases have been so variable that little value can be attached to mortality statistics based upon the reports in the literature.

The method of free incision and exposure to the air with irrigations with solutions of hydrogen peroxide, coupled with amputation when necessary, advocated by Blake and Lahey (31), Cramp (58), and others (351), appears to have given the best results.

A total of 175 cases of emphysematous gangrene and gas phlegmon with a mortality of approximately 45 per cent. has been collected from the literature and are classified according to the mode of entry of the bacillus into the tissues.

Compound fracture, 61 cases.

Arm and forearm, 27 cases; (Thorndike (372), Rothfuchs (2) (305), Hitschmann and Lindenthal (148), Kamen (170), Gildersleeve (106), Klotz and Holman (7) (192), Hewitt (2) (137), Blake and Lahey (2) (31), Cramp (3) (58), Robertson (283), Dunham (71), Clark (445), Jamieson (166), and von Hibler (4) (147)).

Leg and thigh, 34 cases; (Robertson (283), Norris (254), Thorndike (372), Stolz (350), Hitschmann and Lindenthal (148), Klotz and Holman (17) (192), Hewitt (2) (137), Blake and Lahey (2) (31), Cramp (5) (58), White (2) (442), Welch and Flexner (patella) (390)).

Simple fracture, 6 cases.

Arm and hand, 2 cases; (Robertson (283), Westenhoeffer (393)).

Leg and thigh, 4 cases; (Robertson (283), Hitschmann and Lindenthal (2) (148), Muscatello and Gangitano (247)).

Lacerated wounds, 28 cases.

Arm and hands, 9 cases; (Klotz and Holman (192), Hewitt (137), Blake and Lahey (31), Cramp (3) (58), Wolff (399), Werner (428), Pinneo (bite by lion) (450)).

Head and face, 2 cases; (Hitschmann and Lindenthal (148), Blake and Lahey (31)).

Body, 3 cases; (White (442), Welch and Flexner (390) (traumatic rupture of the rectum), Jamieson (166)).

Thigh, leg, and foot, 14 cases; (Klotz and Holman (5) (192), Blake and Lahey (31), Cramp (6) (58), Bill (30), Loving (gored by a caribou) (444)).

Crushing injuries, 21 cases.

Arm and hand, 3 cases; (Spitta (343), Klotz and Holman (192), Welch and Flexner (390)).

Thigh, leg, and foot, 15 cases; (White (442), Welch and Flexner (390), Blake and Lahey (31), Cramp (3) (58), Hewitt (2) (137), Klotz and Holman (5) (192), Cole (55), von Hibler (147)).

Multiple injuries, 3 cases; (d'Agata (8) (2 cases from earthquake in Messina), Hewitt (137)).

Gunshot wounds, 10 cases; (Robertson (283), Stewart (422), Love and Cary (228), Hewitt (137), Cramp (2) (58), White (2) (442), Loving (444), Welch and Flexner (390)).

No previous injury, 4 cases; (Mann (232), Loeb (2) (222), Leroy (429)).

Hypodermic injections, 9 cases; (Soupault and Guillemot (342), Carroll (2 cases, reported by Welch (389)), Dobbin (1 case, reported by Welch (389)), Eagleton (449), Fraenkel (2) (89), Hektoen (430). Anderson (462) has recently reported a case of fatal infection with an organism which was probably *B. welchii*, following injections of snake venom into a patient with epilepsy).

Following surgical operations, 11 cases.

- Opening abscess; (Blake and Lahey (31), Pende (266), Wicklein (395)).
- Ligation of popliteal artery; (Curtis (451), Muscatello and Gangitano (247)).
- Operation for fistula *in ano*; (White (442)).
- Tooth extraction; (Coley (458)).
- Miscellaneous operations; (Cramp (58), Muscatello and Gangitano (247), Hitschmann and Lindenthal (148), von Hibler (147)).

Miscellaneous, 25 cases.

Infection of the lower jaw (Dunham (71)); burn (Cramp (58)); diabetic gangrene (Blake and Lahey (31)); from varicella vesicle (Hallé (122)); snake bite (White (442)); fall from car and from building (2) (Hitschmann and Lindenthal (148)); insect bite (Askanazy (459)), Fraenkel (2) (89), Passow (265), Stolz (350), Ferguson (81), Silberschmidt (337), Sargent and Dudgeon (2) (311), Ferraton (3) (82), von Hibler (5) (147), Martin (469)).

Foamy Organs.

In addition to the above definite infections with the gas bacillus, forty-seven cases of foamy organs (*Schaumorgane*) have been collected from the literature. It is possible that in some of these cases the invasion of the body by *B. welchii* occurred before death, although this was not proved. The list of cases of foamy organs is as follows:

Askanazy (459) (cystitis in a diabetic; necrotic myoma of uterus).....	2 cases.
Reiche (274) (typhoid fever).....	1 case.
Norris (254) (placenta previa; leukemia; hypertrophic cirrhosis of the liver) . .	3 cases.
Pratt and Fulton (271) (rupture of liver by fall; cholotomy for cancer of the bile duct).....	2 cases.
Nicholls (252) (operation for gall stones; inhalation of stomach contents while under anesthesia).....	2 cases.
Westenhoeffer (393) (abortion; Caesarean section).....	2 cases.
Scheidler (325) (forceps delivery; prolonged labor).....	2 cases.
Von Hibler (147) (pleuropneumonia; peritonitis; gastric ulcer with erosion of splenic artery).....	3 cases.
Grassberger (110).....	2 cases.
Picchi (270).....	8 cases.
Goebel (108).....	3 cases.
Sappington (310) (gastric hemorrhage, without discoverable cause).....	1 case.
Howard (160).....	13 cases.
Falkner (79) (gas cysts of the intestines).....	1 case.
Welch and Flexner (390) (purulent infection of the hand; cerebral tumor; gas blebs on the jejunum at autopsy)	2 cases.
Total.....	47 cases.

Infections with Bacillus Welchii without Formation of Gas in the Tissues.

In 1900, in the Shattuck Lecture, Welch (389) called attention to the fact that there might be infection with *B. welchii* without the production of gas in the tissues. He reported the case of an abscess in the neck of a dog from which Harris had isolated *B. welchii*. Pratt and Fulton (271) reported a case in which *B. welchii* was found at autopsy in multiple abscesses of the liver unassociated with the formation of gas in the tissues. Rist (280), Hudson (161), and Turner and Lewis (214, 215, 371) have reported finding *B. welchii* in middle ear or antrum infections, without mentioning the presence of gas. Heyde (435) did not mention gas in the abdominal cavity associated with those cases of appendicitis in which he found *B. welchii* as the predominating organism. Bloodgood (32) mentions a case of infection with *B. welchii* in which there was no formation of gas in the tissues. Spitta (343) isolated *B. welchii* from a case of rapidly spreading gangrene of the hand in which there was no gas production in the tissues. Hosemann (155) has reported a case of infection with *B. welchii* without gas formation, the organism from which did not produce gas in the tissues of a guinea pig. Achalme (3), Thiroloix (356), Rosenthal (295), Savchenko (314), and others have reported finding a similar organism in cases of rheumatism, both before and after death, without mentioning gas production. Rosenow (456) has found *B. welchii* in enlarged lymph nodes which drain joints affected with arthritis deformans. There was no gas in the nodes.

For the successful performance of this function (gas production) by *B. welchii*, the amount of oxygen present is very important. Attention has already been called to the variations in gas production, especially in milk, shown by my own cultures and by the organisms isolated by Stokes and Stoner (348) and by Fraenkel (89, 90).

PATHOLOGY OF INFECTIONS WITH BACILLUS WELCHII.

General.

There is little or no predisposition on the part of the human body to infection with *B. welchii*. Westenhoeffer (394) even claims that *B. welchii* is a pure saprophyte and produces its effects in dead tissues only. For its occurrence unfavorable conditions in the tissues invaded are necessary. Of the 175 cases of gaseous gangrene and gas phlegmon collected above, 139 resulted from such serious, severe injuries to tissues as compound and simple fractures, lacerated wounds, crushing injuries, gunshot wounds, and hypodermoclysis and hypodermic injections, while in only four cases was there no definite history of previous injury.

As pointed out by Kamen (170), the gas bacillus shows a special predilection for certain tissues of the body. As a rule, it either lodges in loose connective tissue (mechanical lodgment) or in the liver or voluntary muscles where it finds a supply of utilizable carbohydrate (glycogen).

B. welchii is able to cause infection either alone or in symbiosis with any of the various pyogenic bacteria. The type of infection will be determined in large measure by the presence or absence of pyogens, and also by the tissue invaded. In general, pure infections with *B. welchii* are associated with an exudate which is remarkably free from pus cells. There is little inflammatory reaction. The organism and its products show a negative chemiotaxis. The meninges furnish an exception to this rule in that in the two cases of gas bacillus meningitis thus far reported (Howard (160) and Hitschmann and Lindenthal (149)) the exudate has been distinctly purulent.

In cases of mixed infections with any one of the pyogens, the reaction of the tissues is more definitely an inflammatory process and the exudate is purulent in character.

Attempts at classification of the lesions produced by *B. welchii* have been made, especially by Muscatello and Gangitano (248) and by d'Agata (8). The former investigators divide these infections into two groups, both of which show as their chief symptoms necrosis and decomposition of the tissues, gas formation, and a tendency to progressive invasion. In group 1 there is a mixed infection; a purulent inflammatory process is present and the tendency to invade healthy tissue is very marked. In group 2 there is pure infection with *B. welchii*, there is no pus formed, and the tendency to invade healthy tissue is not so marked as in mixed infections.

D'Agata (8) also makes two groups of cases. In one, which he calls "gaseous edema," there is a primary necrosis of the tissues without inflammatory changes; there is gas formation in the infected tissues and there are symptoms of general toxemia. In the other group, which he calls "gaseous or emphysematous phlegmon," there are similar symptoms plus an inflammatory reaction in the affected tissues.

The pathologic changes which accompany an infection with *B. welchii* have been so fully described by Hitschmann and Lindenthal (149), Fraenkel (89), Muscatello and Gangitano (248), and others that only a very brief summary is needed here. The infection usually occurs in wounds which have been contaminated with dust or dirt. It often spreads with startling rapidity, by way of the lymph spaces. The patient shows all the symptoms of severe intoxication. The pure infection with *B. welchii* usually

proceeds without the usual signs of inflammation. The first symptom is the appearance of gas in the tissues which one can feel as a fine crepitation. The limb becomes cold, the veins appear as dark lines beneath the skin; the epidermis is raised into bladder-like elevations; and the tissues beneath are infiltrated with a hemorrhagic fluid which has none of the characteristics of pus. Sections of the affected tissues show numerous gas cavities, in the walls of which are many of the bacilli; and the nuclei do not stain. At autopsy there is either only degeneration of the parenchymatous organs, or, if the bacilli entered the blood stream just before death, foamy organs are found.

Experimental.

The experimental work which has been done upon infection of the tissues with *B. welchii* need only be briefly surveyed. Schultze (332) attempted to determine whether *B. welchii* is able to produce gas in living tissue by injecting cultures of the organism into the livers of guinea pigs under anesthesia. In three out of four such animals foamy liver was produced; in one instance the condition certainly originated during life. In these experiments the damage to the liver tissue by the needle, by the pressure of the fluid injected, and by the effect of any toxic substances present in the culture, could not be entirely excluded.

Most experiments to determine the pathogenicity of a given strain are subject to the same criticism. The material injected usually consists of one to three cubic centimeters of strongly acid whey from a milk culture, or of one to three cubic centimeters of a broth culture which contains the products of growth of the bacillus. The acid or metabolic products of the bacteria may produce no little local tissue injury. I have suspended in salt solution the entire growth of *B. welchii* on the surface of two agar "bottle plates" (338) (16 ounce French square bottles), and injected the suspension at once into the peritoneal cavity of a rabbit without producing any observable symptoms.

Muscatello (246), Hitschmann and Lindenthal (149), and Lanier (quoted by Welch (389)) attempted to produce typical gas bacillus infections about recently broken bones in rabbits by the intravenous injection of *B. welchii*. Hitschmann and Lindenthal (149), for instance, found definite gas formation around the site of fracture in twenty-four hours after injection. This rapidly disappeared and no signs were left in three days. Application of a culture of *B. phlegmonis emphysematosæ* (*B. welchii*) to abrasions of the skin or to the stump of an amputated limb did not cause infection.

Rist and Ribadeau-Dumas (282) were able to produce a putrid pleurisy in rabbits by the injection of pure cultures of *B. perfringens* (*B. welchii*).

Norris (254) found that the intravenous injections of pure cultures of *B. welchii* into rabbits were without results; but that the injection of the hemorrhagic fluid from a subcutaneous lesion in another animal was usually fatal.

Ikonnikoff (162) produced an artificial strangulation of the intestine and studied the readiness with which different species of bacteria were able to penetrate the intestinal wall. He found that as long as the wall was not completely necrosed, only certain anaerobes, notably *B. welchii* and *B. paraputrificus*, were able to penetrate. *B. coli* and cocci passed through much later. These results confirm the work of Rocchi (421) and of Garnier and Simon (426), who found *B. welchii* in the blood very early in experimental intestinal occlusion. The latter authors found that within certain limits these bacilli disappeared from the blood as soon as the occlusion was relieved.

Attention has already been called to the work of Heyde (435) who found that anaerobes (among them *B. welchii*) were the first to reach the peritoneal cavity in appendicitis, and that anaerobes were to be found in the margins of an appendiceal abscess. Metchnikoff (238) has reported the results of attempts to produce appendicitis experimentally in chimpanzees. He succeeded in producing the disease only once and that in an animal into whose appendix he had injected a culture of *B. welchii*.

Thirolaix (358) claimed to have produced rheumatic endocarditis and the joint lesions of acute articular rheumatism by the injection of the bacillus of Achalme (*B. welchii*). Rosenthal (296, 297) caused the death of a horse, with symptoms of chorea, by the injection of the same organism.

Loris-Mélikov (225) claims to have produced typhoid-like swelling of Peyer's patches and of the mesenteric lymph nodes by the intraperitoneal injection of cultures of *B. welchii*. If he combined *B. satellitis* with *B. welchii*, he could produce typical typhoid ulceration of Peyer's patches.

ACUTE ARTICULAR RHEUMATISM.

In 1891 Achalme (1) isolated at autopsy from the heart's blood and from the joints of a patient dead of acute articular rheumatism, a bacillus which from his brief description appears to have been identical with *B. welchii*. This report received little or no attention until 1897 when Achalme (2, 3) communicated the results of further study and described other cases of rheumatism in which his bacillus was present. In the same year Triboulet and Coyon (368, 369) reported finding this anaerobic organism in pure culture in the heart's blood of a patient dead of rheumatic endocarditis. About the same time Thirolaix (356, 358) reported five cases of acute articu-

lar rheumatism in which he isolated this same organism from the blood and from the joints during life. He claimed to have been able in each instance to produce in rabbits cardiac, pleuropulmonary, and joint lesions by the injection of the bacilli isolated.

In 1898 Achalme (4) reported finding this same organism on the heart valves in early rheumatic endocarditis. In the same year Savchenko (314) described six cases of acute articular rheumatism, from five of which he obtained the Achalme bacillus, four times in pure culture and once with streptococcus. Bettencourt (28) obtained this organism from blood from a vein at the bend of the elbow of a patient ill with acute articular rheumatism. Carrière (45) isolated this bacillus from the blood and from the pleural effusion of a rheumatic patient during life.

In 1899 Pic and Lesieur (269) reported that they had obtained Achalme's bacillus in blood cultures on two occasions from the same rheumatic patient. Hewlett (139) succeeded in isolating it from a rheumatic knee joint. He compared this strain and one of Achalme's original strains with Klein's *B. enteritidis sporogenes* and found them all identical. It may be noted here that Jackson (164) has shown the identity of *B. enteritidis sporogenes* with *B. welchii*, and that Achalme (5) has shown that his bacillus is identical with the *B. perfringens* of Veillon and Zuber (380).

This line of research on the etiology of acute articular rheumatism appears to have been dropped again until about 1909. In that year Thiroloix and de Bertrand (457) reported that at an autopsy two hours after death, on a patient dying with acute articular rheumatism, they found "the organs affected by the rheumatism packed with the anaerobic bacilli."

Rosenthal (293, 301) has also done much work upon this subject and is convinced that one variety of *B. perfringens*, which he designated as the "*Anhämobacillus*" or the "*variété rhumatismale*" is the cause of acute articular rheumatism. Rosenthal (296) records the interesting fact that a horse which he was immunizing against "the bacillus of rheumatism" died with symptoms of chorea. In this connection it may be noted that Gwyn (118) isolated *B. welchii* repeatedly from the blood of a patient with violent chorea.

In 1913 Achalme (6) again asserted his belief that the bacillus first described by him was the cause of acute articular rheumatism.

Rosenow (456) has recently obtained *B. welchii* in a number of cases from the enlarged lymph glands draining joints showing the lesions of arthritis deformans. The glands were removed with the strictest surgical asepsis.

It may be asked how can an organism whose characteristic lesion is gaseous gangrene produce such a disease as acute or chronic articular rheu-

matism. As analogies one need only think of the variety of lesions produced by the streptococcus, and consider the well known effects of differences in virulence possessed by the different strains of the same organism. Furthermore, attention has already been called to reported cases of infection with *B. welchii*, in which there was no gas formed in the tissues.

In the case of infections with *B. welchii*, however, another factor must be considered; namely, the degree of anaerobiosis present in the medium. My own experiments described above have shown the great effect which differences in the degree of anaerobiosis have upon the reaction of *B. welchii* in milk.

In the case of severe injury to tissues, as in lacerated wounds, compound fractures, etc., there is a sufficient degree of anaerobiosis in the dead tissue to allow the organism to begin to grow and produce its characteristic lesion. By its own products and those of other bacteria present more tissue is damaged or killed and thus the infection spreads. In case *B. welchii* finds itself in living tissue, it may succeed in adapting itself to the amount of oxygen present to such a degree that it is able to live but not able to produce gas gangrene. Under these conditions it is not inconceivable that its pathogenic properties may be so altered that it will produce a lesion as different from gaseous gangrene as erysipelas is from a streptococcic cellulitis.

Bosc and Carrieu (34) have found spores of *B. welchii* on the skin with considerable frequency, and believed that the reported findings of *B. welchii* in the blood have been due to contamination from the skin.

The most important fact, however, which casts doubt upon the claim that *B. welchii* is the cause of rheumatism is the apparent rarity of "foamy organs" at autopsies upon patients dead of this disease. In none of the reported cases of gas in the tissues at autopsy was the cause of death given as rheumatism; and in none of the cases of rheumatism from which this organism was obtained before or after death were foamy organs found at autopsy.

PERNICIOUS ANEMIA.

Herter (132) studied the stools of seventeen patients with pernicious anemia and found *B. welchii* much more numerous than in the stools of normal persons. Blair (quoted by Herter (132)) found that carnivora in the New York Zoölogical Gardens frequently suffer from an anemia of the pernicious type. Herter (132) found *B. welchii* much more abundant in the stools of carnivorous animals than in those of herbivora. Klotz and Holman (192) and Leroy (429) have called attention to the marked anemia

and evidence of the destruction of red blood cells in patients with gaseous gangrene due to *B. welchii*. It is very well known that some strains of *B. welchii* produce hemolysins (see record of experiments above). Schumm (473) found that the serum of patients with bacteriemia due to *B. welchii* gave a spectroscopic test for hemoglobin.

The stools of patients with pernicious anemia examined by me contained higher numbers of spores of *B. welchii* than any others tested.¹ Only one of the patients (Mr. F.) was suffering from diarrhea at the time the examination was made. The technique used will be given later. The results in four cases are shown in Table VIII.

TABLE VIII.

The Number of Spores of Bacillus welchii in the Stools of Four Patients with Pernicious Anemia.

Name.	Date (1913).	No. of spores of <i>B. welchii</i> per gm. of dried feces.	Character of stools.
B. J., female	Aug. 1	4,000,000	Liquid (enema).
	Aug. 19	5,000,000	Liquid, few lumps.
	Aug. 23	625,000	Soft.
Wm. G., male	Aug. 13	7,500,000	Soft.
	Aug. 30	1,000,000	Soft.
F., male	Sept. 5	5,000,000	Liquid.
M. D., male	Aug. 13	2,500,000	Soft.

From the data at hand it is not possible to draw any conclusions as to the relation of the large number of spores of *B. welchii* to the disease itself. Of four strains isolated from stools of patients with pernicious anemia, one produced no hemolysins, two caused traces of hemolysis in the stronger concentration only, while only one produced strong hemolysins (see Table VII, Nos. 23, 28, 29, and 30).

IMMUNITY.

Natural Immunity.

Man possesses a high degree of resistance or natural immunity against *B. welchii*. Welch (389) remarks that "there is good reason to believe that intact tissues of human beings in health possess marked resistance to

¹ For the privilege of examining these patients, I am indebted to the courtesy of Drs. Larrabee and Palfrey, of the Boston City Hospital.

the gas bacillus." Rabbits and mice are also highly resistant to infection with this organism. Pigeons, sparrows, and dogs are somewhat more susceptible. Guinea pigs are quite susceptible to infection by *B. welchii*.

Artificial Immunity.

Fraenkel (89, 91) was unable to protect guinea pigs against infection with *B. phlegmonis emphysematosæ* (*B. welchii*) by injection of bacilli killed either by heat or by chloroform. A guinea pig which had recovered from a gas phlegmon was more susceptible than before. Four to six intraperitoneal injections of dead bacilli in dogs did not protect them against intraperitoneal injection of virulent *B. phlegmonis emphysematosæ* (*B. welchii*).

Klein (182) also found that a previous sublethal dose of *B. enteritidis sporogenes* (*B. welchii*) rendered a guinea pig more susceptible to infection with this organism. The increase in susceptibility was directly proportional to the intensity of the previous infection.

Wild (396) found that guinea pigs which had recovered from an injection of a strain of *B. enteritidis sporogenes* (*B. welchii*) of low virulence or had been injected with heat-killed bacilli, died before control guinea pigs when injected with living *B. enteritidis sporogenes* within a month. Guinea pigs so treated were also killed by the injection of strains which, in the controls, caused only local swelling or ulceration.

Clark (445), on the other hand, reports a case of very serious infection with *B. aerogenes capsulatus* in a human being, treated with an autogenous vaccine. He was of the opinion that the vaccine was responsible for the immediate improvement and later recovery of the patient.

Rosenthal (296, 297, 299) and Rosenthal and Chazarain-Wetzel (302) have attempted to produce a serum against the organism which they claim to be the cause of rheumatism; namely, the bacillus of Achalme (*B. welchii*). The serum of a horse immunized against this organism would protect guinea pigs against the bacillus of Achalme if the injection of the serum were given three to four days previous to the injection of the bacilli. They treated cases of rheumatism with this serum, at the same time using large doses of sodium salicylate, and claimed to have seen slight improvement.

Thirolloix and Rosenthal (360) also used the serum of horses immunized against the bacillus of Achalme (*B. welchii*) and obtained results identical with those reported by Rosenthal and Chazarain-Wetzel (302). They also used vaccines made from this organism in cases of rheumatism, with little or no result. They vaccinated rabbits with the bacillus of Achalme (*B. welchii*) and found that they were protected against intrapleural or intramuscular injections of the living organisms. These results are distinctly

contradictory to those obtained by Fraenkel (91), Klein (182), and Wild (396) in guinea pigs, and can probably be explained by the natural resistance of the rabbit to infection by *B. welchii*.

Phagocytosis.

B. welchii produces substances which cause a negative chemotaxis. It also probably forms leucocidins, as shown by Eissenberg (73) for *B. chauvei* and *B. edematis maligni*. Savchenko (314) found that the exudate from subcutaneous lesions or a culture in muscle extract sterilized by passage through a porcelain filter, exercised a strongly negative chemotactic effect. If a leucocytosis were produced in the peritoneal cavity previous to intraperitoneal injection of virulent *B. welchii* the animal survived; if not, the animal died of the infection. Hosemann (155) noted that the lower the virulence of the strain used the less strong the negative chemotactic influence.

Welch (390), Hitschmann and Lindenthal (149), and Levy (212) have each called attention to the paucity of leucocytes in the exudates in subcutaneous infections with *B. welchii*. In the infections of the meninges by this organism reported by Howard (160) and Hitschmann and Lindenthal (149), and in the abscess in the neck of a dog observed by Harris and reported by Welch (389), the exudate was purulent. Hitschmann and Lindenthal believed that the formation of pus in the meninges was due to the absence of glycogen. The organism, studied by them, which caused a purulent exudate from the meninges caused only a serohemorrhagic exudate in the subcutaneous tissues and muscle.

Fraenkel (89), Kamen (170), and Schultze (332) observed a moderate degree of phagocytosis of *B. welchii* in the experimental lesions in guinea pigs. Hoseman (155) found that the lower the virulence of the strain used the greater the amount of phagocytosis. Guillemot (116) noticed large numbers of the bacilli inside of leucocytes in a case of gaseous edema of the leg.

McCampbell (407) was not able to obtain phagocytosis of *B. welchii* *in vitro* unless the bacilli were washed free from acid which neutralized the opsonins and injured the leucocytes.

Agglutinins.

The reported results of attempts to produce agglutinins for *B. welchii* are very confusing. Kamen (170) was unable to produce an agglutinating serum in rabbits even by the injection of living cultures. Picchi (270) did not succeed in producing agglutinins for *B. welchii* by any method em-

ployed. Klein (182) was unable to detect agglutinins in the blood of animals with fatal and non-fatal infections with *B. enteritidis sporogenes* (*B. welchii*). McCampbell (407), Passini (260), Rocchi (285), and Werner (392), on the other hand, have reported successful attempts in the production of an agglutinating serum.

Bachmann (14) found that the serum of an animal immunized against *B. edematis maligni* would not agglutinate *B. welchii*. Similar negative results were obtained by Leclainche and Vallée (203) with serum of animals immunized against *B. chauvei* and *B. edematis maligni*.

Hewlett (141) states that the serum of some patients infected with *B. welchii* contained agglutinins for this organism. Passini (260) reports positive agglutination tests with *B. welchii* in cases of "characteristic diarrhea." The serum of the same patients also agglutinated *B. putrificus* in dilutions of from 1 to 10 up to 1 to 40. Pic and Lesieur (269) found no agglutinins for the bacillus of Achalme in the serum of patients afflicted with acute articular rheumatism. Herter (132) found that the serum of patients suffering with pernicious anemia did not agglutinate *B. welchii*, and mentioned the work of Theobald Smith, which yielded similar negative results.

The experiments of Passini (260), Rocchi (285), and Werner (392) require special attention.

Passini (260) has accepted the doctrine of Schattenfroh and Grassberger (318, 416) concerning the essential difference between the sporulating and non-sporulating forms of *B. welchii* and the possibility of transforming one into the other. He immunized animals against *B. putrificus*, and against the sporulating and non-sporulating forms of *B. welchii*. He used old cultures killed at 60° to 70° C., because living cultures did not agglutinate well. His results may be summarized as follows: (1) Serum of an animal immunized against *B. putrificus* agglutinated the sporulating form of *B. welchii*, but in lower dilutions than it agglutinated *B. putrificus*. This serum did not agglutinate the non-sporulating form of *B. welchii*. (2) The serum of an animal immunized against the sporulating form of *B. welchii* agglutinated both forms of *B. welchii*, and also *B. putrificus*, but in slightly lower dilutions. (3) The serum of animals immunized against the non-sporulating form of *B. welchii* agglutinated the sporulating form but not *B. putrificus*. In later work Passini (264) speaks of group agglutinations for members of the butyric acid group of bacteria.

Werner (392) immunized four animals against four different strains of *B. welchii* by intravenous injections, at intervals of 6 or 7 days, of suspensions of agar slant cultures killed at 60° C. The serum of each animal

agglutinated its homologous strain but no others, with one exception. The serum of an animal immunized against a gas bacillus isolated from milk agglutinated another gas bacillus isolated from the same sample of milk. One of the cultures used by Werner was one of Fraenkel's original strains.

Rocchi (285) immunized animals against *B. putrificus* (five strains), *B. chauvei*, *B. botulinus*, and several organisms corresponding to *B. welchii*. He was unable to find any "serodiagnostic agreement" among the different species or among the different strains of the same species. A strain of *B. perfringens* (*B. welchii*) from his own laboratory was not agglutinated with the serum of an animal immunized against a strain of *B. perfringens* from Jungano's laboratory, and *vice versa*.

TABLE IX.

The Results of Agglutination Tests with Bacillus welchii.

No.	Serum 2.			Serum 20.			Serum 21.			Serum 22.			Serum 23.		
	1:20	1:40	1:80	1:20	1:40	1:80	1:20	1:40	1:80	1:20	1:40	1:80	1:20	1:40	1:80
2	+	+	+	+	+	-	-	-	-	+	+	-	+	+	-
3	-	-	-	-	-	-	-	-	-	+	-	-	?	-	-
4	+	-	-	+	+	+	-	-	-	-	-	-	+	-	-
9	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
21	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-
22	-	-	-	?	-	-	-	-	-	+	-	-	+	-	-
23a	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
23b	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
25	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
28	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-
29	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-
31	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

An attempt was made to produce agglutinins by the intraperitoneal injections of five rabbits with five different strains of *B. welchii* isolated by me. The results were not satisfactory. It was impossible to produce a serum with a titer greater than 1 to 80. The results obtained with these low titer sera showed great variation. In the case of culture 21 it was not possible to produce agglutinins for either the homologous or any other strain. Even those sera which showed small agglutinating power gave confusing results, as shown in Table IX. To save space in the table, it may be stated here that the following cultures were not agglutinated by any of the five sera: Nos. 1, 5, 6, 7, 17, 19, 20, 24, 26, and 30. Nos. 20 and 21 were not agglu-

minated by their homologous sera. In these tests the macroscopic method was employed and the washed sediment from six day old cultures in plain and sugar-free broth, under oil, was used as the emulsion of bacilli.

McC Campbell (407) immunized rabbits against *B. welchii* and produced in their serum bacteriolysins and precipitins for this organism. He also found that the proteins of *B. welchii* would cause anaphylaxis in guinea pigs. The serum of his immune animals, in the presence of the antigen (*i.e.*, *B. welchii*), would bind complement. Rocchi (285), however, was unable to obtain uniform results in experiments for the fixation of complement. Korentchevsky (195, 196) fed and injected per rectum and intravenously into dogs filtrates from cultures of *B. perfringens* (*B. welchii*) and claimed in each case to have found specific antibodies (precipitins, complement fixators, and agglutinins) in the serum of the animals so treated.

BACILLUS WELCHII IN THE NORMAL GASTRO-INTESTINAL TRACT.

B. welchii is a normal inhabitant of the gastro-intestinal tract of man. The relative numbers of this organism vary in the different age periods, in different individuals, and in the same individual at different times. As a general rule, *B. welchii* is less abundant during the nursing period and most abundant in old age. It is occasionally found in the small intestine, but it is normally limited in its distribution to the cecum and colon.

The First Week of Life.

Flügge (87) in 1894 found *B. butyricus* of Botkin in the feces of breast-fed babies only a few days old. Tissier (361) has made extensive studies of the bacteriology of the intestinal tract in infancy. He described three phases in the process of the establishment of the normal bacterial flora of the intestine of the nursling. During the first few hours of life the intestinal contents are bacteria-free. In a few hours bacteria make their appearance, and for some three or four days in breast-fed, and for a longer period (eight days or more) in bottle-fed babies, the intestinal contents showed many varieties of bacteria. During this period of transition *B. welchii* is prominent among the bacterial flora of the intestine. After the milk diet becomes established, *B. bifidus* and *B. acidophilus* displace the other bacteria.

Passini (258, 264) estimated that there were 1,016 spores of *B. perfringens* (*B. welchii*) per milligram of meconium of a twenty-eight hour old infant which had not received any food. He considered that *B. welchii* formed the greater part of the "characteristic meconium flora."

Sittler (339) and Herter (135) have also reported the presence of *B. welchii* in the meconium of infants.

Table X shows the results of the examination of the stools of infants during the first ten days of life. These stools were obtained from babies born at the Boston Lying-in Hospital.² The stools were received in sterile gauze pads and thus conveyed to the laboratory. The results obtained from these examinations, very incomplete in many cases, confirm the findings, already detailed, of Tissier, Passini, Herter, and Sittler.

It appears from Table X that *B. welchii* is much more numerous in the stools of bottle-fed infants during the first week of life than in those of breast-fed babies.

Strains 12, 20, and 21 of my series were isolated from the stools of three of these infants, Babies Ba., Va., and Co., respectively. Nos. 20 and 21 were typical *B. welchii*, produced strong hemolysins, and were highly pathogenic for guinea pigs. No. 12 resembled *B. welchii* in morphology. It formed spores in sodium chloride solution containing coagulated egg-white, but not in other media, so far as I was able to determine. It produced acid and gas in broth with galactose, glycerin, maltose, mannite, saccharose, and starch, but not in inulin or lactose. It acidified and coagulated milk without the production of gas. This organism was not positively identified. Typical *B. welchii* were also present in the same stool with organism 12.

The fact that such strongly hemolyzing and highly pathogenic strains of *B. welchii* (Nos. 20 and 21) were isolated from the stools of three to six day old infants is important and will be referred to again.

The Nursing Period.

In the intestinal tract of normal breast-fed infants the bacterial flora quickly attain a remarkable degree of uniformity. In bottle-fed babies the uniformity is not so quickly or so thoroughly established (Tissier (361)). The normal flora of the infant's intestine are as follows: in the duodenum and jejunum, *B. aerogenes*; in the lower jejunum and ileum, *Micrococcus ovalis*; in the region of the ileocecal valve, *B. coli* and *B. acidophilus*; and in the transverse and descending colon, *B. bifidus* (Kendall (174)).

B. welchii is present in the gastro-intestinal tract of normal infants during the nursing period. They have been found in such stools by Schatten-

² The specimens were obtained through the courtesy of Drs. McLain and McCann and the nurses of that Institution, to whom I desire to acknowledge my indebtedness.

froh and Grassberger (318), Passini (258), Herter (135), Wollstein (401), Tissier (361), and others. Sittler (339) found *B. welchii* regularly in the stools of nursing infants. Moro (243) considered non-motile butyric acid bacilli among the "constant bacteria" in normal stools of breast-fed babies. Although *B. welchii* is present in the stools of normal nurslings, it is not abundant (Herter (135)). It often can not be detected by the usual heated milk test, probably because in the acid or sugar-containing contents of the intestine it is unable to form spores.

Stools of nineteen babies under one year of age at the Boston City Dispensary were examined for *B. welchii*. The nineteen infants were "feeding cases," none of which showed more than a very slight digestive disturbance. Several of them were apparently quite healthy. None showed pus or blood in the stools. Of these nineteen, eight showed *B. welchii* in the stools. These organisms were not present in abundance, the milk test requiring from forty-eight to seventy-two hours to show stormy fermentation after inoculation with a loopful of the feces and heating to 80° C. for fifteen minutes.

Childhood and Adolescence.

According to Herter (135), there is a slight increase in the number of *B. welchii* in the intestinal tract during this age period.

Adult Life.

B. welchii is universally recognized as a normal inhabitant of the intestines of adults. Passini (258) found *B. welchii* in every adult stool examined by him. Rettger (277) obtained this organism from fifteen out of sixteen adult stools, if as much as thirty-two milligrams of fecal matter was used for the test. Schmidt (464) found Schattenfroh and Grassberger's non-motile butyric acid bacillus regularly in stools. Orton (256) found *B. welchii* in the stools of 83.3 per cent. of those inmates of the Massachusetts State Hospital who had no digestive disturbances, and in 68.6 per cent. of normal persons outside the hospital. Andrewes (13) mentions *B. welchii* as the most abundant of the anaerobes in the intestines of adults.

MacNeal, Latzer, and Kerr (249) attempted to estimate the number of spores of *B. welchii* in the stools of healthy men. The highest number found by them was 39,449 per gram of feces.

According to Herter (135), the number of *B. welchii* in the intestines shows a notable increase in old age.

Distribution of Bacillus welchii in the Intestines.

Sittler (340) believed that *B. welchii* was present in the mucus which lines the wall of the ileum as well as in the large intestine. Herter (135) examined, two hours post mortem, the intestinal contents of a fourteen year old boy, taking specimens from different levels of the canal. He found *B. welchii* in the cecum and colon but not in the small intestine. Moro (243) obtained similar results in the examination of the intestinal contents of four breast-fed babies.

Rocchi (421) noted a great increase in the number of *B. welchii* in the intestinal contents above the point of artificial occlusion of the intestine.

TECHNIQUE.

Many investigators have attempted to devise a method for determining the number of *B. welchii* in both normal and diarrheal stools. Three general methods have been employed: the use of Gram-stained smears; the use of anaerobic plates; the heated milk test. Herter (135) has used extensively the Welch-Nuttall (386) rabbit test.

Klein (181) was unable to develop any reliable method for determining the number of spores of *B. enteritidis sporogenes* in feces.

Most of the methods in use involve heating a suspension of feces to 80° C. for fifteen minutes. This will reveal the presence of spores, but will not show anything of the number of vegetative forms. This is an important fact to be borne in mind in interpreting results and will be referred to again.

Sittler (340) depended upon stained smears. Herter (132), however, found that stained smears were not reliable because of the frequent presence of other bacteria which resembled *B. welchii*.

The making of plates from dilutions of the feces has usually followed heating of the dilution to 80° C. for fifteen minutes. Passini (262) and Herter (132) used this method. Herter found it essential to use blood agar as a medium.

The heated milk test is by far the simplest and is the one used by Glynn (107), Orton (256), Hewes and Kendall (136), Rettger (276), and others. A tube of milk with a thick layer of cream was inoculated with an amount of the specimen of feces, heated to 80° C., and incubated. The occurrence of stormy fermentation was considered to prove the presence of *B. welchii*.

Various methods of estimating the number of spores present from this milk test have been employed. Hewes and Kendall (136) inoculated the milk with a loopful of feces, heated and incubated. If stormy fermentation took place in twenty-four hours, it was considered that many spores

of *B. welchii* were present; if seventy-two hours were required for this to occur there were few spores present. Rettger (277) used loops of various sizes, the largest of which held approximately thirty-two milligrams of feces. Orton (256) used 1, 2, and 3 loopfuls of feces. If stormy fermentation occurred in a tube of milk inoculated with one loopful, many spores were present; if only in the tube of milk receiving three loopfuls, few were present.

Glynn (107) suspended one loopful of feces in one cubic centimeter of sterile water. This dilution was approximately 1 to 1,300. One or more loopfuls of this suspension were inoculated into tubes of milk which were heated and incubated. He also made a suspension of feces in water so that the mixture had a standard specific gravity of 1.005. From this he made further dilutions, inoculating tubes of milk with one cubic centimeter of each.

MacNeal, Latzer, and Kerr (249) have made the most accurate numerical studies of the number of spores of *B. welchii* in stools.

They weighed accurately 2 mg. of feces in a 50 c.c. volumetric flask. This was rubbed up against the sides of the neck of the flask with a sterile rod and 50 c.c. of sterile water were added. The feces adhering to the rod and to the sides of the neck were carefully washed off into the water in the flask and the mixture was thoroughly shaken. The flask was then inverted and any solid particles allowed to collect on the glass stopper. The flask was righted and the fluid allowed to flow out of the neck slowly, leaving these solid particles adhering to its sides. These were again ground with the glass rod. These processes of grinding and shaking were repeated until no solid particles were left to settle on the stopper when the flask was inverted. Dilutions were made from this mixture, heated to 80° C. for fifteen minutes, and 1 c.c. was inoculated into blood agar plates and grown anaerobically. Their results were stated in terms of the number of spores of *B. welchii* per gm. of feces.

This work of MacNeal, Latzer, and Kerr (249) is probably the most accurate yet published on the enumeration of the spores of *B. welchii* in the feces. Its chief source of error is that it can make no allowance for differences in the consistency of the different samples of fecal matter. The method used in my experiments reduced all enumerations to the basis of the number of spores of *B. welchii* per gram of solid matter in the stool. This furnishes a much more accurate basis of comparison, inasmuch as the standard is not influenced by the fluidity or dryness of the specimen of stools.

Blood agar, with or without dextrose, and milk are the media almost universally used for growth and enumeration of *B. welchii* in the stools. Savchenko (314), however, used a mixture composed of three parts of lac-

tose-sodium lactate-broth and one part of milk; and Rodella (288) used a 1 per cent. butyric acid broth and agar.

Herter (135) made use of and recommended very highly the Welch-Nuttall (386) rabbit test as a means of obtaining some idea of the abundance of *B. welchii* in a sample of feces.

He suspended 1 c.c. of feces in 9 c.c. of sterile 0.85 per cent. sodium chloride solution and filtered it through cotton. 1 c.c. of this suspension was injected into the ear vein of a rabbit which was killed one to three minutes afterwards. Differences in the rate and in the amount of gas formation in the body of the rabbit were an index to the number of *B. welchii* present. This method has its sources of error. *B. welchii* has been found in the intestinal tract of normal rabbits. Korentchevsky (196) and Lahey (448) observed a typical Welch-Nuttall reaction in the body of a rabbit which had not been injected with anything.

The technique of my own experiments was as follows:

0.5 gm. of feces was weighed into a 50 c.c. volumetric flask. Into this was measured 50 c.c. of sterile water, making a dilution of 1 to 100. This was then placed in a shaking machine and shaken vigorously for fifteen minutes. If any solid particles were still visible, the shaking was repeated. Further dilutions up to 1 to 5,000, and in some cases even up to 1 to 1,000,000, were made, and 1 c.c. of each dilution, including the 1 to 100, was inoculated into tubes of milk and heated to 80° C. for fifteen minutes. These were incubated for from twenty-four to seventy-two hours, no result being called negative until after seventy-two hours' incubation. Tubes showing stormy fermentation of the milk with the production of the odor of butyric acid, stained smears from which showed bacilli of characteristic morphology, were called positive. In many instances the organism was isolated for further study.

25 c.c. of the 1 to 100 dilution were placed in an evaporating dish, previously accurately weighed, and evaporated to dryness over the water bath. The dish was then placed in a desiccator for several hours (usually over night) and again weighed. The total solids in 25 c.c. of the 1 to 100 dilution and the highest dilution, 1 c.c. from which gave a positive reaction, being thus known, it was an easy matter to calculate the minimum number of spores per gram of dried feces (Table XI). This method, while somewhat complicated yields more reliable results than any yet used in estimating the number of spores of this organism in the stools.

It became necessary, in order to give this method full value, to determine the number of spores of *B. welchii* required to produce the reaction of stormy fermentation in milk. 1 c.c. of each dilution was inoculated simultaneously into a tube of milk and a tube of dextrose agar, respectively, and each heated in the same water bath to 80°C. for fifteen minutes. The agar tubes were immediately poured into plates containing a few drops of sterile defibrinated blood and grown anaerobically in Novy jars after the manner already described for the isolation of *B. welchii*. The milk tubes were incubated as usual.

TABLE XI.

The Number of Spores of Bacillus welchii in Stools of a Healthy Man.

Date (1913).	Highest dilution giving a positive test.	Mg. of dried feces in 25 c.c. of 1:100 dilution.	No. of spores of <i>B. welchii</i> per gm. of dried feces.	Character of stool.
July 30	Undiluted*			Normal; formed.
July 31	1 : 100	29	860	Normal; formed.
Aug. 1	1 : 100	125	200	Hard.
Aug. 2	Undiluted*			Normal; formed.
Aug. 4	Undiluted*			Normal; formed.
Aug. 5	Undiluted*			Normal; formed.
Aug. 6	1 : 100	27	925	Normal; soft.
Aug. 12	1 : 100	34	740	Normal; soft.
Aug. 13	Undiluted*			Normal; formed.
Aug. 20	1 : 100	28	890	Normal; soft.
Aug. 21	1 : 100	60	420	Normal; soft; formed.
Aug. 21	1 : 1000	16	15,600	Liquid; effect of cathartic.
Sept. 4	1 : 100	55	450	Hard.
Sept. 8	1 : 100	12	2,100	Liquid; cathartic.

* Loopful of feces inoculated into a tube of milk, heated to 80°C. for fifteen minutes and incubated.

From Table XII it is seen that a dilution which yields two to three colonies on anaerobic dextrose blood agar plates invariably gave positive results in milk.

TABLE XII.

The Relation between the Number of Colonies of Bacillus welchii on Dextrose-Blood Agar Anaerobic Plates and Stormy Fermentation of Milk.

	No. of colonies on dextrose- blood agar anaerobic plates.							Stormy fermentation in milk.						
	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400
Feces. (S. P.) Dec. 15.....	0	1	0	0	0	0	0	+	-	-	+	+	-	-
Feces. (S. P.) Dec. 18.....	0	0	0	0	0	0	0	+	+	+	+	+	+	-
No. 1 in sterilized suspension of feces.....	5	2	0	1	0	0	0	+	+	+	-	-	-	-
No. 6 in sterilized suspension of feces.....	15	12	5	1	1	0	0	+	+	+	+	+	+	-
No. 19 in sterilized suspension of feces.....	8	3	1	0	1	0	0	+	+	+	+	-	-	-

In most instances where there was one colony in the plates, the milk tube inoculated from the same dilution was also positive. In one case there were no colonies on the plates, but the corresponding tube of milk showed a positive result. Hence, while it seems a legitimate conclusion that a dilution of feces which will show one or more colonies on blood agar will also be capable of bringing about stormy fermentation in milk, it seemed best to state the results obtained by this method as "the minimum number of spores in one gram of dried feces."

BACILLUS WELCHII AND TYPHOID FEVER.

Klein (182) examined the stools of forty-four typhoid patients for *B. enteritidis sporogenes* (*B. welchii*). In the diarrheal stages of the disease, he found this organism very abundant. In the absence of diarrhea and during convalescence they were present in much smaller numbers or were entirely absent from the stools.

Loris-Mélikov (225) has made a special study of the relation of *B. welchii* to typhoid fever. In those cases with severe gastro-intestinal symptoms, spores of *B. welchii* were very numerous in the stools in spite of the fact that, as he claims, diarrhea always tends to the elimination of anaerobes. In cases with severe toxic symptoms and less gastro-intestinal disturbances, spores of *B. welchii* were few in number in the stools. In the former instance, *B. welchii* was associated with another anaerobe which the author called *B. satellitis*. He believes that these two organisms play a part in the necrosis and ulceration of Peyer's patches and in some of the complications of typhoid fever, especially perforation and hemorrhage.

One strain of *B. welchii* isolated from a typhoid stool by Loris-Mélikov was exceedingly virulent for guinea pigs, killing the animals in four to eight hours after intraperitoneal injection. He claims to have been able to produce marked swelling of Peyer's patches by the intraperitoneal injection of *B. typhosus* and *B. welchii*, and to have produced typical typhoid ulceration of Peyer's patches by the injection of *B. typhosus*, *B. welchii*, and *B. satellitis*. He was able to produce ulceration only when *B. satellitis* was used.

My own studies of *B. welchii* in typhoid or typhoid-like infections have been limited to two cases. One liquid stool from a man whose clinical history was not available showed stormy fermentation of milk sixteen hours after being inoculated with one loopful of feces and heated to 80° C. for fifteen minutes. Organism 4 of my series was isolated from this case. It was not pathogenic for guinea pigs. From the same stool *B. typhosus* was isolated on Endo plates.

The second case was that of a fourteen year old girl who showed symptoms of a mild typhoid fever. The Widal test was negative, and no typhoid bacilli were obtained from cultures from the clot of blood taken for a Widal test nor from the stools. The most abundant aerobic organism in the stools was a bacillus which was identical culturally with *B. alcaligenes*. This bacillus agglutinated with the patient's serum in a dilution of 1 to 40. The stools were not diarrheal. From one of them a typical *B. welchii* was isolated, which was exceedingly virulent for guinea pigs. Intraperitoneal injection of two cubic centimeters of a forty-eight hour broth culture killed the animal in five hours. In the thin, serosanguineous exudate, there was with *B. welchii* an aerobic organism resembling *B. coli*. Although no quantitative tests for *B. welchii* were made on the stools of this patient, the organism appeared to be present in small or moderate numbers only.

BACILLUS WELCHII AND INTESTINAL PUTREFACTION.

The relation of *Bacillus welchii* to intestinal putrefaction is by no means clear. The confusion which exists with reference to the ability of *B. welchii* to split proteid has already been discussed. Herter (135), Metchnikoff (236), Loris-Mélikov (224), and Tissier (365, 366) class *B. welchii* among the putrefactive intestinal bacteria. Rettger (276) and others (see Lotti (227), Schattenfroh and Grassberger (319), Distaso (65)) think that *B. welchii* is preeminently a fermentative organism and capable of attacking proteid only very slightly, if at all.

Herter (135) stated that intestinal indicanuria was evidence of intestinal putrefaction. It was usually easy to produce this condition by feeding dogs large quantities of meat. In such cases he found "anywhere in the colon or the lower ileum, moderate or considerable numbers of anaerobic, spore-forming, butyric-acid-producing bacteria as well as colon bacilli." One is led to believe, from the further discussion, that *B. welchii* made up the majority of these anaerobic bacteria, and that the indicanuria was believed to be largely the result of their activity. He explains in a similar manner the headache, flatulence, and increase of indican in the urine which follows the imperfect action of a cathartic in some persons. The action of the cathartic causes the passage of native proteids and possibly the peptones from the small into the large intestine where the former is attacked by anaerobes if they are present. In persons who harbor large numbers of *B. putrificus* and *B. welchii*, such an occurrence would result in the appearance of considerable indican in the urine.

The mere fact of the increase in the number of sporulating *B. welchii* under the conditions just mentioned might easily be explained by the fact

that these are just the conditions under which *B. welchii* sporulates most freely. It does not prove the relation of *B. welchii* to the production of indol in the intestine. While some strains of *B. welchii* do form indol, the great majority of these which have been isolated from feces and elsewhere do not produce indol.

Herter (135) also believed that *B. welchii* was largely responsible for a specific type of intestinal putrefaction which he called "saccharobutyric." "This form of intestinal derangement is characterized by a chronic putrefactive process (having its seat mainly in the large intestine and lower ileum) and due to the action of very large numbers of strictly anaerobic butyric-acid-producing bacteria capable of multiplying by means of spore formation. . . . The organism most prominently concerned in at least a large number of cases is *B. aerogenes capsulatus* (*B. welchii*). Associated with *B. aerogenes capsulatus* may be found *B. putreficus*." In blood agar plates from suspensions of the feces heated to 80° C. for fifteen minutes, *B. aerogenes capsulatus* was the dominant or even exclusive anaerobe. The stools of such patients are soft, light in color (due to the reduction of bilirubin), of low specific gravity (due to the gas formation), and have the odor of butyric acid. The softness of the movements, he thought probably due, in part, to ammonium butyrate which is formed in considerable amount and acts as an irritant to the intestine.

"Chronic excessive saccharobutyric intestinal putrefaction" was found by Herter to be quite wide-spread among adults and especially among elderly persons. Clinically this condition is characterized by an irritability of the digestive tract, with a tendency to desquamation of the epithelium of the mouth and probably throughout the entire length of the intestinal canal (134). Carbohydrates are badly borne, excessive intestinal flatulence and sometimes slight diarrhea often following the use of considerable amounts of cereals and starchy foods. There is also a slowly developing anemia of the secondary type in most cases. Some of these patients become confirmed invalids.

Distaso (64) believed that constipation was due largely to the effect of toxins formed in and absorbed from the large intestine as a result of the growth of bacteria. Among the bacteria mentioned by him as having part in this process was *B. welchii* which he classed as indol-former. Friedman (431) found that intestinal stasis (constipation) was accompanied by an increase in the number of *B. welchii* (in the sporulating form). Tissier (365) found that the stools of persons whose large intestine harbored large numbers of *B. welchii* became compact when the carbohydrates of the diet were reduced to a minimum and proteids greatly increased.

Metchnikoff (237, 238) is especially convinced of the relation of *B. welchii* to intestinal putrefaction. He believes that the number of bacteria in the intestinal tract is a source of danger to the host. There are three anaerobes, commonly found in the intestines, which, according to Metchnikoff, produce powerfully toxic substances; namely, *B. putrificus*, *B. sporogenes*, and *B. welchii*. Of the three, *B. welchii* is the most active producer of toxic substances. Each of these organisms, Metchnikoff states, form indol, skatol, and phenol, which, absorbed from the intestine, produce grave lesions of important organs such as arteries, liver, and kidneys.

BACILLUS WELCHII AND DIARRHEA.

In Adults.

Klein (179) in 1895 first isolated *B. enteritidis sporogenes* (*B. welchii*) from the stools of patients affected in two epidemics of diarrhea which occurred in St. Bartholomew's Hospital, London. In the first epidemic there were 59 cases (180); in the second, 144 cases (181). Andrewes (10) found the same organism in the stools of patients in St. Bartholomew's Hospital in another epidemic of 44 cases, and in 11 out of 20 unselected cases of diarrhea. Hewlett (138) isolated it from the stools of 12 patients with ulcerative colitis, from the stool of one with ordinary diarrhea, and from that of one with chronic dysentery. Klein (181) found *B. enteritidis sporogenes* (*B. welchii*) in the feces in 6 out of 8 cases of cholera nostras.

The above mentioned cases all occurred in adults. The symptoms in the acute cases were mild. None of them terminated fatally. In Klein's cases, the earliest symptom was abdominal pain, followed in about half an hour by diarrhea. There were from two to eight movements in twelve hours. The stools were watery, brownish yellow in color, and contained much mucus, and, in the severest cases, streaks of blood. Vomiting was very rare. Recovery usually occurred in twelve hours.

According to Andrewes (9), *B. enteritidis sporogenes* (*B. welchii*) was usually limited to the milder, but sometimes chronic forms of diarrhea; being absent, as a rule, from the stools in diarrhea of a choleraic character.

Schattenfroh and Grassberger (318) were of the opinion that *B. enteritidis sporogenes* had nothing to do with the diarrhea in Klein's cases because he (Klein) found that same organism in nine out of thirteen samples of milk examined. Although Klein (180) believed that the infection in the outbreaks described was carried by milk, no infections resulted from the use of milk from the same source as the nine samples in which he found the bacillus.

Hewlett (138) found *B. enteritidis sporogenes* in the stools of eleven out of thirteen healthy persons examined. He concluded that "it is quite conceivable that in diarrhea, etc., the number of organisms present and their virulence might be greater than under normal conditions without the diseased condition being necessarily due to the activity of the bacteria."

Glynn (107) after examining the evidence very carefully and making numerous studies of his own, declared that "there is no satisfactory evidence that *B. enteritidis sporogenes* is a cause of acute or epidemic diarrhea." For (1) he found spores of this organism in considerable numbers in the stools of normal persons; (2) there was no difference in the virulence of organisms from diarrheal and from normal stools; and (3), as a rule, all organisms normally inhabiting the intestines tend to multiply excessively when the character of the stool is modified by disease. Glynn even ingested cultures of *B. enteritidis sporogenes* without producing any noticeable effects.

Savage (312) states that "it has now been established that *B. enteritidis sporogenes* had nothing to do with the outbreak from which Klein first isolated it."

Romanovitch (291) found *B. perfringens* (*B. welchii*) in great numbers in the stools of a man suffering with amebic dysentery. The number of these bacilli remained high after recovery from the dysentery. Lotti (227) studied two cases of diarrhea and two of constipation, in all of which *B. welchii* was present in large numbers in the stools.

Herter (135) believed that the tendency to diarrhea seen in persons with the saccharobutyric form of intestinal putrefaction was due to the presence of ammonium butyrate, and that the acid from which this was formed was produced by the gas bacillus and related anaerobes. In these cases the stools were light in color, of low specific gravity, and acid in reaction. There was much flatulence, and carbohydrates were tolerated badly.

Hewes and Kendall (136) have reported four cases of diarrhea in adults in which they thought *B. welchii* played an important part. Two were cases of acute diarrhea, and two of chronic colitis in elderly persons. In the two acute cases, the onset was sudden; the diarrhea was severe with pus, mucus, and blood in the stools; and there was some fever. Their reasons for associating *B. welchii* with the diarrhea in these cases were: (1) the presence of excessive numbers of the spores of the gas bacillus in the stools (a positive reaction in twenty-four hours or less in milk inoculated with one loopful of stool and heated to 80° C.), with reduction in the number of spores after treatment (either a negative result or forty-eight to seventy-two hours required for positive reaction in milk); and (2) all four persons showed a marked improvement on a "pure proteid and buttermilk diet," and became worse on a carbohydrate diet.

The occurrence of spores of *B. welchii* in the stools of healthy adults has already been discussed. In Table XI is given a series of estimations of the number of spores of this organism in the stool of a healthy adult over a period of more than a month. The number varied from 200 to 925 per gram of dried feces, except after a cathartic. In the liquid cathartic stool there were over 15,000 spores of *B. welchii* per gram of dried feces. The increase to this number was sudden. With the return to normal in the character of the stool there was a fall in the number of spores, although the decrease was, in this instance, not as rapid as the increase had been. This change is in harmony with the statement of Glynn (107) already quoted, but contradicts that of Loris-Mélikov (225) who states that a condition of diarrhea tends to cause the disappearance of anaerobic bacteria from the stools, and of Friedman (431) who claimed that the number of *B. welchii* in the stools was greatest in a state of constipation.

TABLE XIII.

The Number of Spores of Bacillus welchii in Stools of a Male Adult with Diarrhea.

Patient.	Date.	Highest dilution giving positive test.	Mgm. of dried feces in 25 c.c. of 1:100 dilution.	No. of spores of <i>B. welchii</i> per gm. of dried feces.	Character of stool.
F. M. Adult	July 25	1 : 500	85	3,000	Normal.
	Aug. 6	1 : 100,000	35	714,000	Soft, diarrhea.
	Aug. 13	1 : 1,000	18	13,900	Liquid, much mucus.
	Aug. 14	1 : 10,000	11	1,136,000	Liquid, mucus, and undigested food.
	Sept. 3	1 : 1,000	54	4,630	Normal, formed.
	Sept. 7	1 : 5,000	31	80,600	Soft.

In Table XIII are shown the results of a series of examinations of the stools of an adult who suffered an attack of diarrhea during the period of examination. At the beginning of the examination his stools contained about 3,000 spores of *Bacillus welchii* per gram of dried feces. Two weeks later he developed a diarrhea which lasted about ten days. The stools were liquid, not frothy, brown in color, and contained much mucus and sometimes undigested food. There was never any blood in the specimens examined. Aerobic cultures yielded very great numbers of a bacillus resembling *Bacillus proteus*. The number of spores of *B. welchii* rose to more than a million per gram of dried feces. After recovery from the diarrhea the number of spores fell to 4,630 per gram of dried feces, to rise to 80,000 four days later when the bowels became loose again. This

patient did not appear to be especially upset by carbohydrates before, during, or after the attack of diarrhea.

A discussion and attempted explanation of the above facts will be made further on.

In Children.

Klein (181) in 1897 reported finding *B. enteritidis sporogenes* (*B. welchii*) in the stools of eleven infants suffering with diarrhea.

Passini (264) believed that the "dyspepsia" which is frequently seen in infants when they begin to receive milk is purely "physiologic." He found *B. welchii* to be the predominant organism in meconium. As soon as the milk with its sugar reached the intestines, *B. welchii* began to produce acid. This acid caused increased peristalsis with the resultant looseness of the bowels. As soon as *B. welchii* was replaced by *B. bifidus* the "dyspepsia" ceased. Passini was also convinced that the "*blaue Bacilliose*" of Escherich (468) and others was due to the abundance of *B. welchii* in the stools, and that the type of diarrhea described by Escherich as associated with this phenomenon was a true gas bacillus diarrhea. Intestinal disturbances due to *B. welchii* were much greater in nurslings than in adults because of the presence of sugar in the intestinal contents of the former.

Lehmann (208) spoke of *B. enteritidis sporogenes* (*B. welchii*) as a "specific cause of disease" ("*spezifischer Krankheitserreger*") during the first year of life.

Kühl (200) asserted that *B. coli* and *B. perfringens* (*B. welchii*) predominate in the intestinal flora of children in the first year of life only in cases of intestinal irritation and intestinal catarrh.

Sittler (339) has claimed that in slight digestive disturbances, *B. aerogenes* and *B. coli* are the most abundant organisms in the stools, while in severer forms *B. coli* and *B. welchii* predominate. The constant association of *B. coli* with *B. welchii* in cases of "enterocatarrh" suggested to Sittler a symbiotic relation between the two. He thought that the action of the putrefactive (*i.e.*, "sporulating") form of *B. welchii* in association with *B. coli* would account for certain symptoms of enterocatarrh, such as the fever and intoxication. In a digestive upset, bacteria which were normally few in number might multiply unduly (*e.g.*, *B. welchii*). This overgrowth of *B. welchii* in symbiosis with *B. coli* might cause even more serious disturbances. On account of this symbiosis *B. welchii* would be able to grow in places in which it would not otherwise be capable of growing.

In a later report Sittler (340) again emphasizes the pathologic possibilities of the symbiotic growth of *B. welchii* and *B. coli*. He believed

further that the presence of an inflammatory exudate, produced according to Finkelstein's (436) idea of "alimentary intoxication," was especially favorable to the growth of *B. welchii* and *B. coli*, but not of other organisms. He thought also that under certain conditions *B. welchii* might acquire the power of forming toxic products in the intestinal contents.

Korentchevsky (194) has asserted that although *B. welchii* is a normal inhabitant of the human intestine, its pathologic influence sometimes increases and grave intestinal disturbances result.

Veeder, Kilduffe, and Denny (379) examined the stools of 100 infants in Philadelphia suffering from diarrhea and found *B. welchii* in only one. This work appears to have been done in the summer of 1911, the season before the "gas bacillus year" in Boston (175). Kendall's work would indicate that *B. welchii* is only very rarely present in the stools of infants with dysentery due either to *B. dysenteriae* or to streptococcus. This may account for the very low percentage of positive tests for *B. welchii* obtained by Veeder, Kilduffe, and Denny (379) in their series of cases. As might be expected, the gas bacillus is present in considerable numbers in the stools of adults afflicted with bacillary dysentery. Thus Orten (256) found *B. welchii* in 73.3 per cent. of the stools from patients in the Worcester State Hospital who were infected with *B. dysenteriae*.

Tissier (362) has described a specific clinical type of diarrhea in infants due to *B. perfringens* (*B. welchii*). In his opinion this organism is the chief if not the sole cause of summer diarrhea in breast-fed babies. Tissier describes the disease in great detail and divides its clinical manifestations into three periods: the onset; the fully established disease; and the terminal period. The symptoms differ somewhat in breast- and bottle-fed infants.

In the breast-fed children the onset occupies two to six days. The normal stools of the previously healthy baby, little by little become harder; darker in color, and of a slightly fetid odor. After a day or two, the stools become more frequent, liquid, yellowish in color, and their passage is accompanied by the emission of abundant gas. The child is less playful, cries frequently, its appetite is less, and it will not take the breast well.

After the disease has become established there are from six to twelve stools a day. These are thick, yellowish brown in color, contain yellowish gray lumps, and are rendered foamy by the abundant gas they contain. Left in the air they change rapidly to an olive green color. Usually two to four of the stools in a day are abundant, the others less so. There is colic at the time of the passage of the stool. Excoriation of the buttocks is very frequent even in the best cared for children. Sometimes there is

a vesicular eruption on the forehead, face, and other parts of the body. There is rapid loss of weight. This condition persists for from fifteen to twenty days.

The improvement is gradual and slow, sometimes requiring from fifteen to thirty days for complete recovery. The stools become less frequent and less foamy, and become mucous and glairy. The appetite improves, the eruption disappears, and the weight increases. Slight exacerbations lasting two to three days are not infrequent. Finally, the stools rather suddenly become golden yellow and the child is well again.

In breast-fed infants the disease runs a course of one to two months. After apparent recovery a slight error in diet may cause an immediate reappearance of symptoms. The prognosis is generally good, Tissier having never seen a fatal case in a breast-fed child.

In bottle-fed babies, on the other hand, the onset is sudden, the symptoms reaching their height in two to three days. The stools rapidly increase in number up to ten or twelve in twenty-four hours. They are liquid, greenish yellow at first, but become olive green on exposure to the air, foamy, and slightly fetid. There is rapid loss of weight. The temperature sometimes rises to 38° or 38.5°C. The prognosis is grave. One of Tissier's patients died on the eighth day of the disease.

Bacteriologic examination of the stools of such patients showed that *B. bifidus* had almost entirely disappeared. In all his observations on these cases Tissier found that besides *B. coli* and *B. enterococcus* (vestiges of the normal intestinal flora), the only organisms constantly present were *B. perfringens* (*B. welchii*) and Bacillus III of Rodella (467).

Tissier reports the case of a healthy three months' old child whose stools were free from *B. welchii*. The mother began to nurse, twice daily, another infant, suffering with the disease above described. In eight days after the mother began nursing the second child her own previously healthy baby became ill and showed clinical symptoms and bacteriological findings identical with those of the second infant.

Tissier has attempted to answer the question: Under what conditions is *B. perfringens* (*B. welchii*) able to displace *B. bifidus* in the intestine of an infant? He states that in sugar media *B. bifidus* produces sufficient acid to arrest the growth of *B. welchii*, *B. coli*, etc. But in liquid media deprived of sugar *B. welchii* is able to arrest the growth of *B. bifidus*, while *B. coli* and *B. enterococcus* can grow in its presence. This arrest of the growth of *B. bifidus*, Tissier asserts, is not due to simple overgrowth of *B. welchii* in the broth, because grown separately both organisms show about the same rate of growth; but it is probably due to some substance secreted

by *B. welchii*. Hence, Tissier concludes, *B. perfringens* (*B. welchii*) is not able to develop in the presence of the normal intestinal flora of an infant unless the chemical constitution of the intestinal contents is modified and unless the proportion of carbohydrates is greatly diminished. In the treatment of such cases, therefore, Tissier feeds sugars. While the facts detailed by Tissier can not be disputed, his interpretation of them is not to be accepted.

The feeding of sugars in intestinal infections with the gas bacillus is absolutely contra-indicated according to Kendall (176), who has done the most important work in this country on this type of infection. Kendall (173) has emphasized the fact that "the very severe, acute summer diarrheas of bacterial causation present a constant syndrome consisting of prostration and fever associated with mucus, pus, and frequently blood in the movements. . . . Bacteriologically considered these cases are of varied etiology and caused by organisms of unlike characters." He has noted an interesting cyclic variation in the bacteriology of the cases of diarrhea which have occurred among the patients of the Boston Floating Hospital during the past four years. "One year the dysentery bacillus was the dominant type met with; the second year streptococci were conspicuous; the third summer was noteworthy because of the great number of cases in which the gas bacillus was the predominant organism encountered;" while in the fourth summer an entirely different organism made its appearance. In each instance, the type of infection which showed itself the following year appeared occasionally among the cases received toward the end of the season.

Kendall and Smith (176), at the Boston Floating Hospital in 1910, found 22 cases (7 per cent.) with gas bacillus out of 293 cases of diarrhea. The following year Kendall, Day, and Bagg (174) found this organism in 33 (12 per cent.) out of 283 cases of diarrhea, while in 1912, out of 135 cases of diarrhea 35 (26 per cent.) showed the gas bacillus (175). They call attention to the epidemic character of gas bacillus diarrhea during the summer of 1912 as contrasted with 1910 and 1911.

According to Kendall and Day (175), two factors are necessary to the multiplication of *B. welchii* in the intestine: (1) an excess of utilizable carbohydrate; and (2) a deficiency of organisms in the intestinal tract capable of forming lactic acid from this carbohydrate in sufficient volume and concentration to inhibit the growth of the gas bacillus which is "rather sensitive to lactic acid." The gas bacillus forms butyric acid which acts as an irritant, causing diarrhea even to the extent of mucus, blood, and pus in the stools. Those milk tests which are positive in eighteen to twenty-

four hours are more significant than those which require a longer time. Of 103 cases of so called "fat diarrheas," which, like gas bacillus diarrheas, do poorly on carbohydrates, only six showed definitely large numbers of *B. welchii*.

Among the ninety cases of diarrhea associated with *B. welchii* observed by Kendall and his coworkers in three years there were four deaths, all from complications not associated with the gastro-intestinal tract.

This type of intestinal infection is made quickly and definitely worse by the feeding of sugars. Hence the treatment should consist of the feeding of butter-milk either alone or better with lactic acid bacilli (Kendall and Day (175)). Clock (52) has reported especially favorable results from the feeding of lactic acid bacilli in a variety of diarrheas.

TABLE XIV.

The Number of Spores of Bacillus welchii in Stools of Two Infants with Diarrhea.

Patient.	Date.	Highest dilution giving positive test.	Mgm. of dried feces in 25 c.c. of 1:100 dilution.	No. of spores of <i>B. welchii</i> per gm. of dried feces.	Character of stools.
Baby P.	Aug. 19	1 : 10	25	1,000	Pus and mucus.
Baby P.	Aug. 20	1 : 5,000	61	205,000	Pus and blood.
Baby P.	Aug. 23	1 : 10,000	31	807,000	Pus and mucus.
Baby P.	Aug. 26	Negative in undiluted solutions			Mucus.
Baby M.	Aug. 20	1 : 10,000	4	12,500,000	Pus and mucus.
Baby M.	Aug. 26	Negative in undiluted solutions		*	Mucus.

The summer of 1913 was not a "gas bacillus year" in Boston. There were no typical cases of diarrhea due to *B. welchii* at the Boston City Dispensary during that time. A few of the children examined showed small numbers of *B. welchii* in the stools; that is, a tube of milk inoculated with a loopful of undiluted feces gave a positive reaction in forty-eight to seventy-two hours. Two babies showed the organism in considerable numbers, as shown in Table XIV.

The specimens of feces of the two babies referred to in Table XIV, as well as those from others at the Boston City Dispensary, were obtained by means of a rectal tube. The examinations were made within two hours after the specimen was obtained.

The enormous number of spores of *B. welchii* in the stools of Baby M., the rapid improvement and sudden decrease in the number of spores after feeding butter-milk is probably sufficient evidence to warrant classing this case as one of gas bacillus diarrhea. In the case of Baby P., however, the comparatively small number of spores of *B. welchii* in the stools at the beginning of the attack, their slow increase in numbers, the failure to show definite improvement on a butter-milk diet, the presence of streptococci in great abundance, and the fatal termination after *B. welchii* had apparently disappeared, are sufficient to exclude this case from the gas bacillus infections.

ATTEMPTS TO PRODUCE EXPERIMENTAL DIARRHEA BY FEEDING BACILLUS WELCHII.

Numerous attempts have been made to produce diarrhea experimentally by feeding cultures of *B. welchii*, but usually with little success. Glynn (107), who, from an examination of the evidence did not believe that *B. enteritidis sporogenes* (*B. welchii*) had anything to do with diarrhea, himself ingested great numbers of the bacilli without ill effect. The character of the diet at the time of the experiment was not stated.

Hewlett (138) fed this organism to monkeys without being able to induce a condition of diarrhea.

Jacqué (165) fed *B. welchii* to adult guinea pigs without any ill effects. When fed to very young guinea pigs in milk, however, it caused distension of the abdomen with gas and retardation of growth. One guinea pig which he began feeding on the fifth day of life died on the tenth day (fifth after beginning the feeding of *B. welchii*). It lost weight rapidly and the temperature fell from 37° to 34.5°C. At autopsy the stomach and intestines were distended with gas and contained foul-smelling material. *B. welchii*, in the vegetative form, was isolated from the contents of the stomach and small and large intestines, but no spores were found. Jacqué suggests injecting *B. welchii* into the mammary gland of the mother to test its pathogenicity when ingested by the young animal.

Passini (264) fed to dogs the filtrate of cultures in his dextrose beef-trypsin-digest medium and produced vomiting. When introduced through a fistula into the upper small intestine, it caused violent, painful diarrhea from which the animal usually recovered in an hour or so. This was followed in ten to fourteen days in many cases by pronounced hemorrhagic catarrh of the mucous membrane of the intestine, sometimes involving the entire length of the intestinal tract.

Tissier (362) fed two litters of young kittens for ten and twenty days, respectively, on cow's milk, and then fed pure cultures of *B. perfringens* (*B. welchii*). In twenty-four hours diarrhea began. One kitten emaciated rapidly; the stools were frequent, liquid, greenish yellow in color, and frothy. This animal died in five days. Autopsy showed great numbers of *B. perfringens* (*B. welchii*) in the intestinal contents.

Metchnikoff (237) fed *B. welchii* to chimpanzees without being able to produce diarrhea in these animals.

Korentchevsky (195, 196) has reported the results of feeding to young animals cultures of *B. welchii* isolated from the same species. This was done on the supposition that an organism which might be pathogenic for man might not be so for any other animal, and *vice versa*. He found that while he was unable to produce intestinal lesions in a dog or rabbit by feeding an organism isolated from human feces, he could produce pathologic conditions by feeding cultures isolated from the feces of a dog or a rabbit, respectively. He fed groups of puppies on a diet of bread and beef to which was added every one to four days half a liter of a culture of *B. welchii* in a mixture of chopped beef and water. After the first week the culture was not eaten well. Those puppies receiving *B. welchii* showed slow loss of weight, sometimes the stools were fluid and foul, and sugar agar inoculated with the feces and heated to 80° C. for fifteen minutes yielded pure cultures of *B. perfringens* (*B. welchii*). Even in similar cultures from the stools of normal puppies, however, he obtained a greater number of *B. welchii* than of any other anaerobic organism. Two of the puppies fed upon *B. welchii* died. They showed at autopsy general emaciation, anemia, and fatty degeneration of the kidneys and liver.

With the exception of feeding milk cultures of *B. welchii* (from human stools) to three guinea pigs and two rabbits without being able to produce diarrhea, my experiments were limited to the feeding of cultures of *B. welchii* to two full grown monkeys. The results are shown in Table XV. All the cultures used were of proved virulence for guinea pigs, except Nos. 27 and 32. Their virulence was not tested. It was not possible to get the monkeys to take the cultures in milk. An attempt was made to feed the cultures in hard boiled eggs, but the suspicions of the animals caused them to refuse this unusually generous partiality shown them in the matter of diet. By injecting the cultures into unpeeled bananas with a Luer syringe the monkeys ate them without suspicion.

From Table XV it is seen that it was not possible to produce in adult monkeys a definite diarrhea by the feeding of *B. welchii*. It is possible that the age of the animals may have affected the results. Two interest-

TABLE XV.
The Effects of Feeding Bacillus welchii to Monkeys.

Monkey.	Date.	Feeding.	Character of stool.	No. of spores of <i>B. welchii</i> per gm. of dried feces.
Rhesus 167	Aug. 29	Usual diet (bananas)	Formed, normal	48,000
	Sept. 8	Sediment from broth culture 19* in banana		
	Sept. 9	Whey from milk culture 22* in banana		
	A.M.	Whey from milk culture 27* in banana		
	Sept. 10		Soft	Negative.**
	P.M.	Whey from milk culture 6* in banana		
	Sept. 10		Acid; soft; 5 or 6 a day	Negative.**
	P.M.			
	Sept. 10		Acid; soft; 5 or 6 a day	860
	A.M.	48 hr. "bottle-plate" culture 22 in banana		
Java	Sept. 11		Acid; soft; semi-formed	Negative.**
	P.M.			
	Sept. 11		Formed; normal	Negative.**
	Sept. 12	Usual diet (bananas)	Formed; normal	5,700
	Aug. 29	Usual diet (bananas)	Formed; hard	430
	Sept. 8	Sediment from broth culture 21 in banana		
	Sept. 9	Whey from milk culture 22 in banana		
	A.M.	Whey from milk culture 32 in banana		
	Sept. 10		Formed; hard	Negative.**
	P.M.	Whey from milk culture 6 in banana		
	Sept. 10		No stool since morning	
	A.M.	48 hr. "bottle-plate" culture 22 in banana		
	Sept. 11		Formed; hard	13,500
	P.M.			
	Sept. 11	Usual diet (bananas)	Formed; hard	12,500
	Sept. 12	Usual diet (bananas)	Formed; hard	19,000

* The strains used were as follows:

No. 6. From a normal appendix obtained at autopsy; virulent.

No. 19. From a stool of an infant with diarrhea; virulent.

No. 21. From a stool of a normal six day old infant; virulent.

No. 22. From a stool of an adult with diarrhea; very virulent.

No. 27. From cow feces.

No. 32. From a stool of an adult with pernicious anemia.

** Negative = no stormy fermentation in milk inoculated with a loopful of undiluted feces, heated to 80° C. for fifteen minutes, and incubated for seventy-two hours.

ing facts are, however, shown in Table XV. First, although the stools of *rhesus* 167 contained a larger number of spores of *B. welchii* than those of the Java monkey on the first preliminary examination, during the experiment the condition was reversed. That is, the stools of the Java monkey, which showed a distinct tendency to constipation, contained larger numbers of the spores of *B. welchii* after the feeding began than those of *rhesus* 167. In the second place, on the third day of the feeding the stools of *rhesus* 167 became more numerous, softer, and more strongly acid, but the number of spores of *B. welchii* was very low. Three out of the four specimens examined during this period gave negative results when a large loopful of the stool was inoculated into milk and heated to 80° C. for fifteen minutes.

This last mentioned phenomenon is not so surprising when it is remembered: (1) that patients suffering with gas bacillus diarrhea do poorly on a carbohydrate from which *B. welchii* produces all, or the greater part, of the irritating substances which cause intestinal irritation and excite increased peristalsis; and (2) that it is under just these conditions, *i.e.*, in the presence of utilizable carbohydrate, that *B. welchii* does not form spores in artificial culture media. In other words, if it is in the so called putrefactive form (as distinguished from the so called fermentative form) that *B. welchii* causes diarrhea, one would rather expect that in cases of true gas bacillus diarrhea the number of spores of *B. welchii* in the stools would be fewer during the height of the attack than later on when the carbohydrate diet had been changed to a "pure proteid" diet. For in the latter instance, *i.e.*, in a sugar-free medium, conditions are favorable, if we may judge conditions in the intestines by the results in the test-tube, for the sporulation of *B. welchii*. This point and the results of experiments made in an attempt to elucidate it will be discussed a little later.

INFLUENCE OF DIET UPON THE PRESENCE AND ACTIVITY OF BACILLUS WELCHII IN THE INTESTINES.

Sittler (339) has asserted that the feeding of levulose or saccharose to infants causes digestive disturbances in which *B. welchii* displaces *B. bifidus*. He was unable to confirm this statement with test-tube experiments. Maltose and lactose, he claimed, favored the growth of *B. bifidus* rather than *B. welchii* (340). Too much fat in the diet yielded fatty stools and constipation with *B. welchii* as the predominating organism. Bahrdt and Beifeld (16) also found that increase of fat in the milk caused an increase of mucus and of the number of *B. welchii* in the stool. These statements are not in harmony with the experiments of Kendall and Day (175),

who found *B. welchii* in only a small per cent. of the cases of so called "fat diarrheas."

Hartje (126) was unable to confirm Sittler's statement that the feeding of cane sugar resulted in the displacement of *B. bifidus* by *B. welchii* in the stools. He thought that the decrease in the number of *B. bifidus* was due to the smaller amount of sugar in the large intestine on a cane-sugar diet. Hartje studied the effect of feeding different kinds of sugars in various concentrations upon the sugar content and reaction of the stools. He found that the administration of 7 per cent. sugar solution yielded measurable amounts of sugar in the stools, the smallest amount being found after feeding beet-sugar, larger amounts after lactose, and the largest amounts after feeding malt extract.

Passini (264) has reported finding sugar throughout the entire intestinal tract of nurslings, and that certain cases of intestinal disturbances show great improvement upon the withdrawal of sugar from the diet.

Wollstein (401) has studied the stools of infants on different diets and found spores of *B. welchii* much more numerous on a proteid than on a carbohydrate diet.

The work of Hewes and Kendall (136) and of Kendall and Day (175) upon the effect of diet upon gas bacillus diarrhea has already been referred to. They found that both children and adults with diarrhea whose stools showed excessive numbers of gas bacilli were made worse by feeding sugars, and that such patients promptly improve when the carbohydrate diet is changed to one of "pure proteid" and buttermilk.

Morse (244) reports like results in similar cases. He found maltose especially bad because of the readiness with which it undergoes butyric acid fermentation.

Tissier (365) believes that the presence of carbohydrate causes increased vitality of *B. perfringens* (*B. welchii*) and augments its digestive activity for proteids. This, he thinks, explains the genesis of certain gastro-intestinal troubles seen in certain patients who are given a diet rather rich in carbohydrates and more or less mixed with milk and eggs. It causes flatulence, colic, and soft frothy stools accompanied by the violent emission of gas. In the stools of these patients *B. welchii* is especially abundant. A change of diet to meat, or eggs and meat, causes a rapid cessation of symptoms.

Smith (465) has described two types of diarrhea in children. One is the well known form due to *B. dysenteriae*, associated with ulceration of the intestine, and best treated by the administration of solutions of sugar, after an initial purge. The other type is associated with the presence of

large numbers of *B. welchii* in the stools. Pathologically, "no ulcers are found, but there is a general pin-point exudate over the mucous membrane of the large intestine. In the severe lesions a pseudomembrane is formed." The gross character of the stools in the two types does not differ greatly. But patients with the gas bacillus form are made worse by the administration of sugars. The feeding of a 5 per cent. solution of lactose in one case caused a rise in temperature of more than 3 degrees (341).

DISCUSSION OF RESULTS.

B. welchii is a normal inhabitant of the intestines of adults and is sometimes found in the stools of infants, but in the latter always in small numbers only. It may be found in the adult intestine in great numbers under a variety of conditions. Spores of this organism are almost invariably present in great abundance in the stools of persons with pernicious anemia. When examinations of the stools of the same person are made at frequent intervals it is found that there are frequent marked fluctuations in the number of spores present; that during a period of constipation there is sometimes an increase and sometimes a decrease; and that as a result of a cathartic there may be a pronounced increase in the number of spores in the stools. The reason for this is probably to be found in the daily variations in diet.

B. welchii is said to be the cause of a fairly definite type of diarrhea, especially in infants. What is the evidence upon which this claim is based?

(1) In such cases, spores of the organism are found in excessive numbers in the stools. While the methods used in determining this have necessarily been crude, they probably give a fairly accurate idea of the approximate number of spores present. While different strains of *B. welchii* show considerable variation in the rate of growth, it is a permissible conclusion that a loopful of feces which gives a positive reaction in heated milk in eighteen to twenty-four hours contains a greater number of spores of *B. welchii* than a loopful which requires forty-eight to seventy-two hours to develop a positive reaction in milk. But in interpreting the result of such a test two things must be borne in mind: (a) that as shown by MacNeal, Latzer, and Kerr (249) and by Sittler (340) the number of spores in different parts of the same fecal mass may show great variation; and (b) that the degree of anaerobiosis in the tube of milk will influence both the speed of the reaction and the character of it. If milk which has been standing for more than a week is used, heating to 80° C. for fifteen minutes will not always produce a sufficient degree of anaerobiosis for the best growth of *B. welchii*. Either the production of stormy fermentation will require a longer time, or

the reaction will not be typical, only acid, and coagulation without gas formation being the result.

(2) Patients with diarrhea associated with excessive numbers of gas bacilli in the stools are made worse by feeding them a diet rich in carbohydrates, and they show improvement when the diet is changed to one of pure proteid with or without buttermilk.

The arguments against the claim that *B. welchii* is causally related to the diarrhea with which it is frequently found associated, may be stated and answered as follows:

(1) *B. welchii* is constantly present in the stools of healthy adults and may be present in small numbers in the normal stools of healthy infants. Variations in individual susceptibility explain the failure to cause diarrhea in some of these cases; variations in pathogenicity of different strains of the organisms explain its harmlessness in others; while absence of those conditions of diet, etc., which favor its assuming a pathogenic part explains its failure to produce diarrhea in other cases.

(2) *B. welchii* is present in unusual abundance in the meconium when the intestine of the infant is entirely without defense. In this case the promptness with which the defense, in the form of the normal nursing intestinal flora, is established saves the child from disaster.

(3) In children given cow's milk from birth the meconial flora, of which *B. welchii* is usually the predominant organism, is slow in disappearing, from fifteen to thirty days being sometimes required. But during this period *B. bifidus* and other protective organisms are present and are gradually getting the upper hand. If bottle-fed babies are given mother's milk for the first 8 days of life, *B. welchii* is not afterwards found under normal conditions (Tissier (362)).

There are certain theoretical considerations which should be discussed at this point. Since patients with excessive numbers of *B. welchii* in their stools are made worse by feeding carbohydrates, and since, as Kendall and Day (175) have pointed out, one of the factors necessary to the multiplication of *B. welchii* in the intestine is the presence of an excess of utilizable carbohydrate, it would seem a warranted conclusion that it is as an actively fermenting organism and by the production of acids that *B. welchii* produces its pathologic effects in the intestine. It is also true that in the presence of fermentable substances or of free acids *B. welchii* does not produce spores in artificial media. It would seem, therefore, that one might expect, *a priori*, that instead of there being an increase in the number of spores of *B. welchii* in the stools in gas bacillus diarrhea there should in reality be a decrease, because the conditions in the intestines at this time

are similar to those under which, in artificial media, *B. welchii* can not be made to sporulate. Furthermore, it would seem also, that one might expect, *a priori*, that a pure proteid diet would bring about conditions favorable to sporulation by *B. welchii* and that the number of spores of the organism would actually show an increase as the patient improved, for most strains of *B. welchii* sporulate fairly well in an alkaline medium in the absence of fermentable carbohydrate.

Experiments were undertaken to determine, if possible, why the reverse of what might be expected is actually found to take place. In the first place, it was thought possible that, as Sittler (340) claimed, *B. welchii*

TABLE XVI.

The Effect of the Presence of Acid upon Spore Formation by Bacillus welchii in Sterilized Suspensions of Feces, Alone and in Symbiosis with Bacillus coli and Bacillus subtilis.

Culture No.	In symbiosis with	Period of incubation.	1 % n/1 sodium hydroxide.	Neutral.	1 % n/1 hydrochloric acid.	2 % n/1 hydrochloric acid.	3 % n/1 hydrochloric acid.	1 % n/1 sodium chloride.	Neutral.	1 % n/1 acetic acid.	2 % n/1 acetic acid.	3 % n/1 acetic acid.	Time required for positive reaction.
			dys.										
19	<i>B. coli</i>	4	+	+	—	—	—	+	+	—	—	—	72
19		4	+	+	?	—	—	+	+	—	—	—	72
19		<i>B. subtilis</i>	4						+	+	—	—	—
20	<i>B. coli</i>	5	+	+	—	—	—						48
20		5	+	+	—	—	—						48
21		7	+	+	—	—	—						48
21	<i>B. coli</i>	7	+	+	—	—	—						48
26	<i>B. subtilis</i>	2						+	+	—	—	—	72
26		2						+	+	—	—	—	72

might sporulate in the presence of free acid if grown in a suspension of feces in symbiosis with *B. coli*. Accordingly, a suspension of feces was prepared, rendered exactly neutral to phenolphthalein, and sterilized in large tubes under oil. One set of tubes was rendered alkaline by the addition of 1 per cent. sterile $\frac{N}{1}$ sodium hydrate. Two other sets were rendered 1, 2, and 3 per cent. acid to $\frac{N}{1}$ acetic and hydrochloric acids, respectively. Four different organisms were tested for spores after growth from two to seven days in these media either alone or in symbiosis with *B. coli* and *B. subtilis*. The results are shown in Table XVI. The growth of *B. coli* was proved by subsequent aerobic cultures, and of *B. subtilis* by the

formation of a scum immediately underneath the layer of oil. From Table XVI it is seen that *B. welchii* does not sporulate in the presence of 1 per cent. or more of free acid either alone or in symbiosis with *B. coli* or *B. subtilis*. These results are entirely out of accord with those of Sittler already referred to (339).

An attempt was next made to determine the effect, if any, upon the sporulating powers of *B. welchii* of the whole fecal flora and of the presence of various sugars in the presence of the mixed flora. A suspension

TABLE XVII.

The Effect of the Presence of Sugars upon the Sporulation of Bacillus welchii in Unsterilized Suspensions of Feces in Sterile Salt Solution.

Patient and date (1914).	Character of specimen of fecal suspension.	Time in incubator at 37°C. Anaerobic.	Terminal acidity (phenolphthalein).	Dilutions and spores.							
				1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800
S. P. Jan. 26	Fresh	hrs.		+	+	+	+	+			
	Plain	24	0.4	+	+	+	+	+	+	+	+
	With 1% lactose	24	3.0	+	+	+	+	+	+	+	—
S. P. Feb. 3	Fresh			+	+	+	—	—			
	Plain	24	0.6	+	+	+	+	+	+	+	+
	With 1% dextrose	24	5.4	+	?	+	—	—	—	—	—
	With 1% lactose	24	5.4	+	+	—	+	—	—	—	—
	With 1% maltose	24	4.4	+	+	+	—	—	—	—	—
S. P. Feb. 24	Fresh			+	—	—	—	—	—	—	—
	Plain	48	0.4	+	+	+	+	+	+	+	+
	With 1% lactose	48	2.8	+	+	+	+	+	+	+	+
	With 1% maltose	48	2.8	+	+	+	+	+	+	+	+
	With 1% saccharose	48	2.8	+	+	+	+	+	+	—	+

+ = presence of spores shown by the occurrence of stormy fermentation in milk after inoculating with 1 c.c. of the dilution, heating to 80° C., and incubating.

— = absence of spores shown by failure of stormy fermentation.

in sterile salt solution was made on several occasions from the stools of a person known to carry spores of *B. welchii*. The typical results of three such tests are shown in Table XVII.

Tubes of milk were immediately inoculated with 1 c.c. of each of a number of dilutions to determine the highest which, in the fresh state, would give a positive reaction in milk heated to 80° C. for fifteen minutes. The suspension was then distributed in 50 c.c. tubes. One of these in each lot was left plain, while to the others were added

enough of a sterile 10 per cent. solution of different sugars to make a 1 per cent. solution. These tubes were then set in a Novy jar over pyrogalllic acid and sodium hydroxide solution and strongly exhausted with the vacuum pump. After twenty-four to forty-eight hours' incubation the contents of the different tubes were thoroughly mixed and 1 c.c. of each of a number of dilutions inoculated into milk and heated to 80° C. for fifteen minutes to determine the highest dilution which would give a positive test for *B. welchii*. These results were then compared with those obtained for the corresponding fresh specimen. The results on the three different dates are not to be compared with each other since no special effort was made to prepare suspensions of uniform density.

From Table XVII three interesting facts are apparent:

(1) That in the plain suspension of feces without sugar and kept under anaerobic conditions in the incubator for twenty-four to forty-eight hours, there was a pronounced increase in the number of spores of *B. welchii*.

(2) That in the presence of the mixed fecal flora, *B. welchii* may sporulate even in the presence of free acid and fermentable sugar.

(3) That the character of the associated flora is an important factor in determining the amount of sporulation. When acid formers are abundant so that the acidity of the medium is raised to 4 per cent. or more of acid to phenolphthalein, there is no increase in the number of spores after twenty-four hours' incubation under anaerobic conditions. When, on the other hand, acid producers are absent or few in number, so that the acidity of the medium does not exceed 3 per cent. to phenolphthalein, *B. welchii* is able to produce spores in abundance. That this ability to sporulate depends upon symbiotic relations of *B. welchii* with one or more species other than *B. coli* is shown by the negative results in the attempts to induce sporulation in fecal suspensions of varying degrees of acidity, as shown in Table XVI.

How then shall we explain (1) the sudden increase in the number of spores of *B. welchii* in the stools after the administration of a cathartic, as shown in Table XI; and (2) the excessive number of spores of *B. welchii* in the stools in cases of so called gas bacillus diarrhea, whose beginning is associated with a diet rich in carbohydrates?

It may be that the cathartic loosens from the walls of the intestine and mixes with the contents, some of the mucus in which *B. welchii* is probably, and according to Sittler, actually, present in considerable numbers under normal conditions. It is more likely, however, that the effect of the cathartic is to sweep down sufficient amounts of sugar or other carbohydrates to render the contents of the lower ileum and the cecum especially favorable to the growth of *B. welchii*. Thus a larger number of vegetative forms are swept into the lower part of the intestinal tract where conditions are favorable for sporulation.

In the case of gas bacillus diarrhea the presence of an excess of carbohydrates in the intestinal contents brings about conditions in the lower ileum and first part of the colon which are particularly conducive to the growth of *B. welchii*. The absence of lactic acid-producing bacteria, as pointed out by Kendall (173) and as indicated experimentally in Table XVII, render conditions still more favorable to the multiplication of these organisms. They, therefore, rapidly increase in numbers, produce irritating butyric acid, and are swept on in excessive numbers into the lower bowel. Here the acidity of the intestinal contents has been greatly reduced by absorption (Hartje (126)). But even in the presence of a moderate degree of acidity, in the presence of mixed fecal flora poor in lactic acid producers, *B. welchii* is able to sporulate rapidly and in abundance. The number of spores produced will be measurably proportional to the number of vegetative forms of the bacillus which reach this part of the bowel; hence the excessive number of spores of *B. welchii* in the stools in cases of gas bacillus diarrhea.

When the diet is changed from carbohydrate to pure proteid one of the factors essential to the excessive multiplication of *B. welchii*, namely, the presence of excess of carbohydrate, is removed. If buttermilk is given, the second essential to the abundant growth of this organism, namely, the absence of lactic acid-producing bacteria, is eliminated by the actual introduction of such bacteria into the intestine. In a field in which competition among bacterial species is so keen as it is in the human intestine, a less violent change of conditions than the substitution of a pure proteid and buttermilk diet for one rich in carbohydrates, is sufficient to change completely the relative numbers of the different species present. Hence it is not surprising that after such a change of diet there should be a very sudden decrease in the number of spores of *B. welchii* in the stools.

SUMMARY.

1. It has been pointed out that the various names, *B. welchii*, *B. phlegmonis emphysematosæ*, *B. enteritidis sporogenes*, *B. perfringens*, *Granulobacillus saccharobutyricus immobilis*, the bacillus of Achalme, *B. emphysematis vaginæ*, and one of the organisms described by Sanfelice, probably represent the same species of bacteria.

2. *B. welchii* probably designates not a fixed species but a closely related group of bacteria which should be further classified.

3. A tentative basis of such classification within the group is suggested by the ability of the different strains to produce acid and gas or to sporulate in media containing inulin and glycerin.

4. The cultural and biological characteristics and the toxin-producing and hemolyzing powers of about fifty strains of *B. welchii* isolated from various sources have been studied more or less completely.

5. A method for determining the number of spores of *B. welchii* in the stools has been described.

6. Quantitative studies have been made of the number of spores of *B. welchii* in the stools of normal healthy adults, of infants during the first week of life, of adults and infants with diarrhea, and of patients with pernicious anemia.

7. It has been demonstrated that in symbiosis with the mixed fecal flora in suspensions of feces, *B. welchii* is able to sporulate even in the presence of fermentable carbohydrate, provided the character of the flora is such that the acidity of the mixture does not rise above 3 per cent. of $\frac{N}{T}$ acid, phenolphthalein being the indicator.

8. Finally, it has been shown that the number of spores of *B. welchii* in the stools is a reasonably accurate index of the number of actively fermenting, disease-producing organisms of this type higher up in the intestine.

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